MSc Thesis – defended in July 2025

Continuous-flow Column Experiments for Enhanced Biotic and Abiotic Degradation of cis-DCE in Limestone

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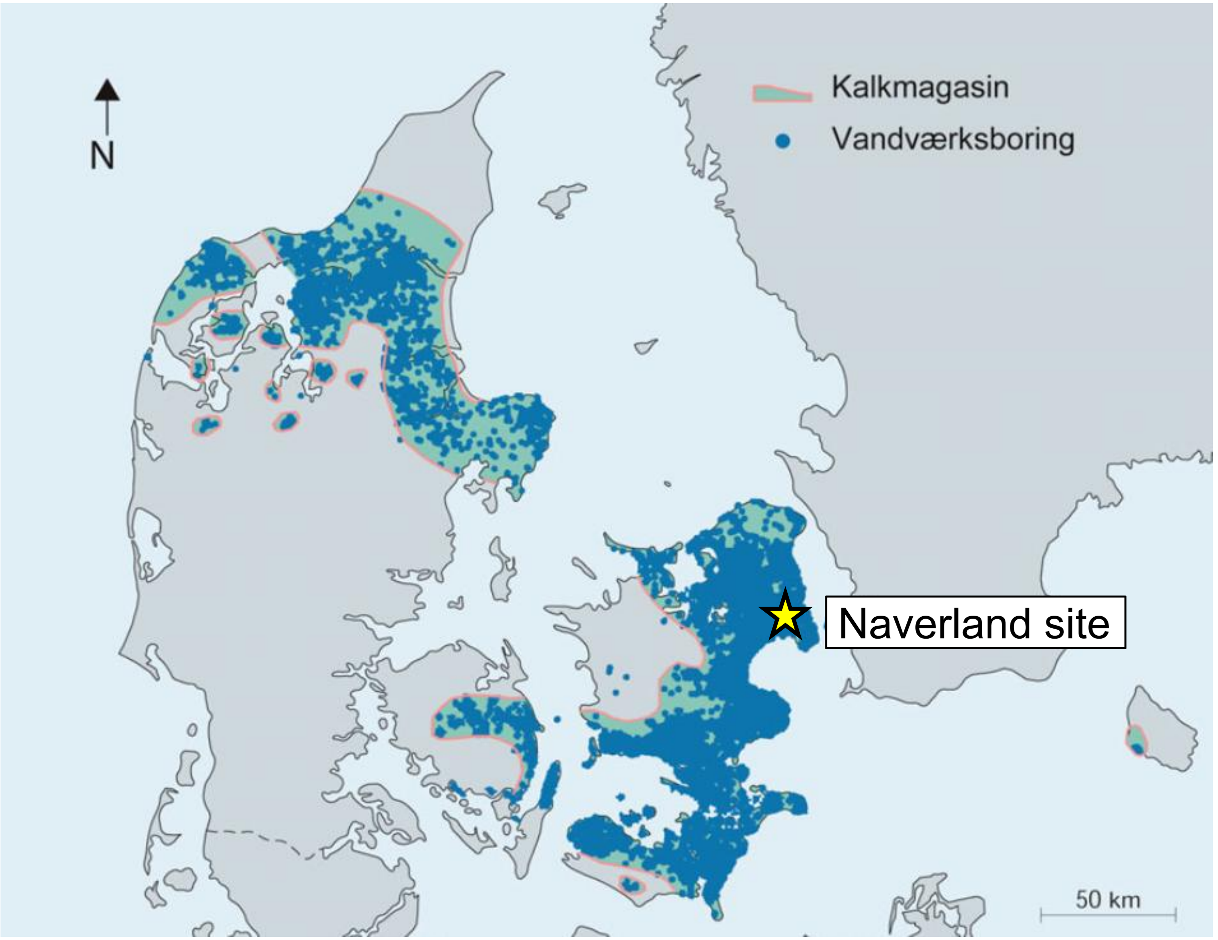
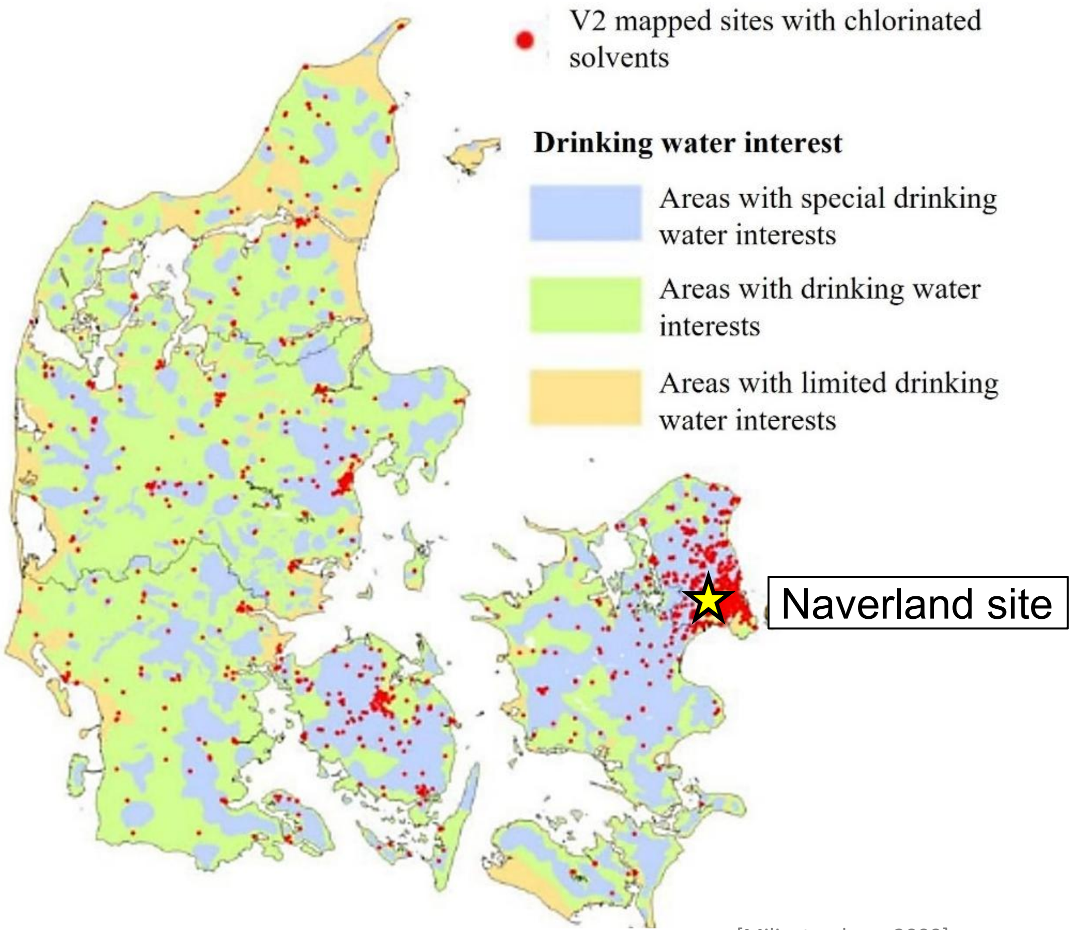
Drinking water in Denmark

Limestone aquifers



[Sonnenborg, 2006]

Chlorinated solvents contamination

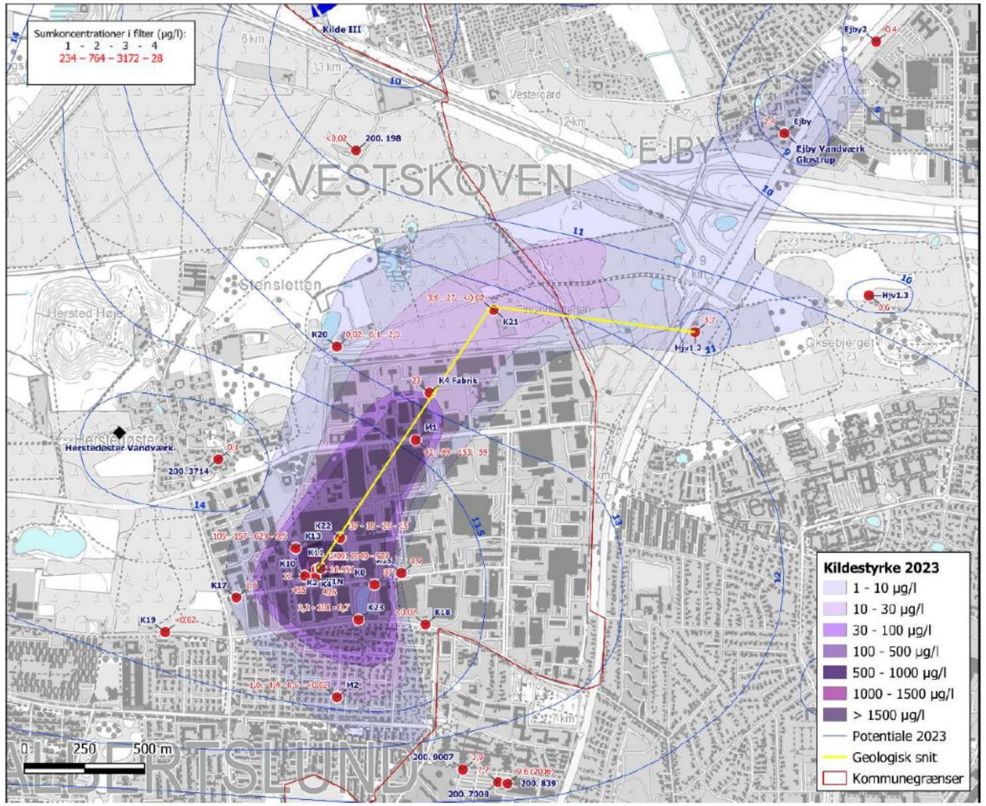


[Miljøstyrelsen, 2009]

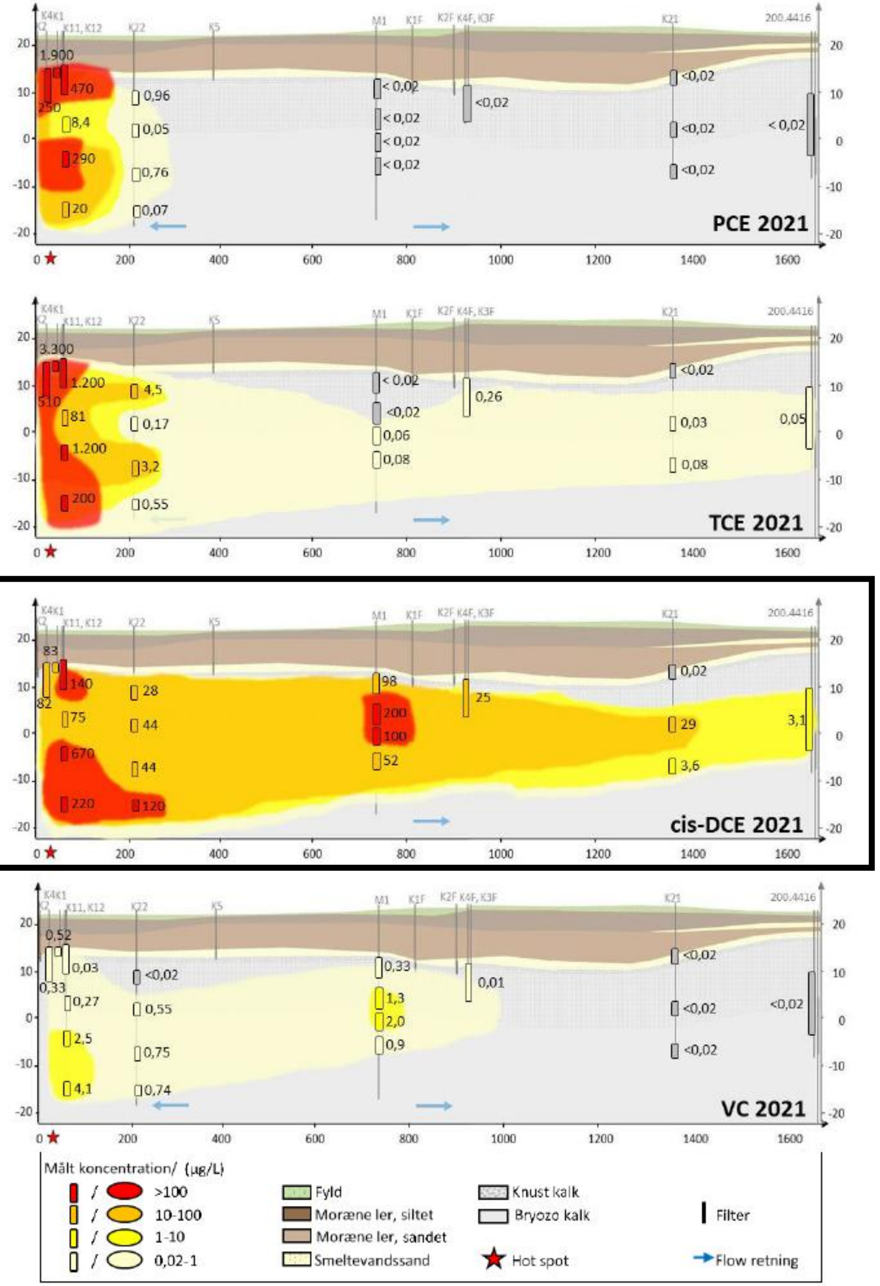
Naverland site

Drinking water quality criteria [1]:

- 1 µg/L for PCE, TCE, 1,1-DCE, 1,2-DCE
- 0.2 µg/L for VC



[HOFOR, 2024]

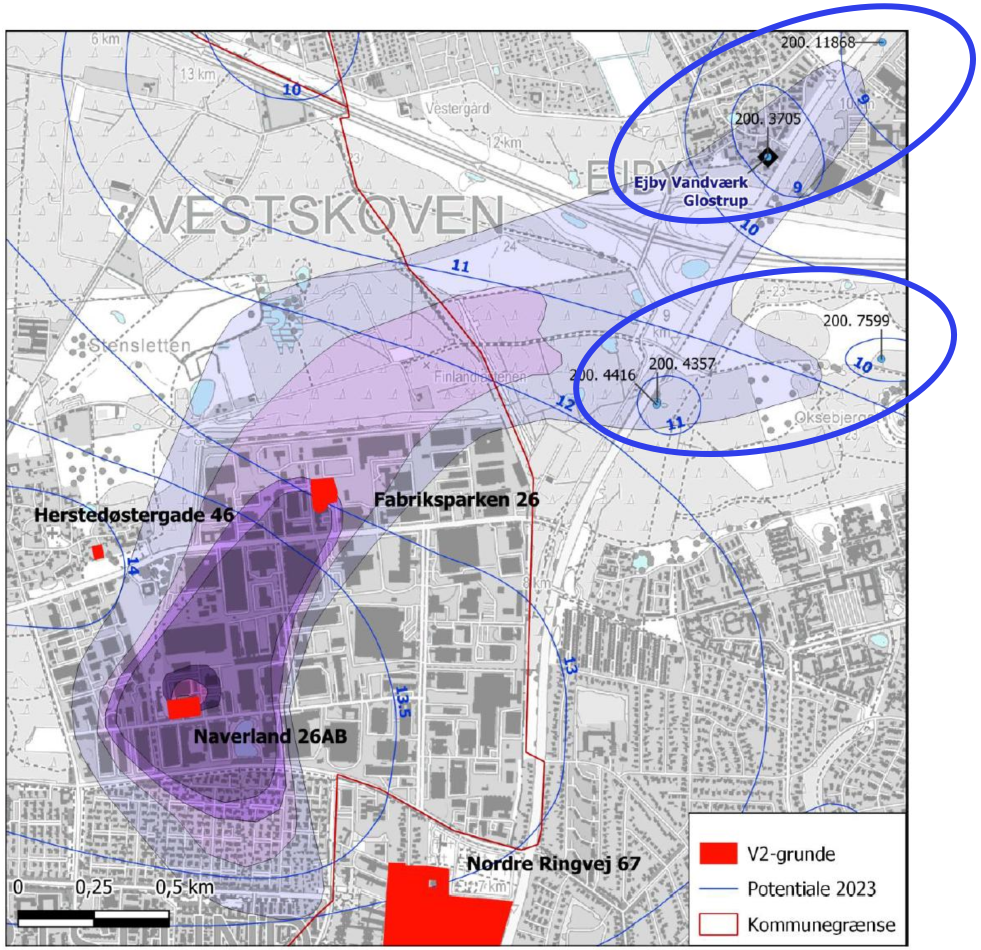


cDCE dominant in the plume

[HOFOR, 2024]

(1) Miljøministeriet (July 2021). *Liste over kvalitetskriterier i relation til forurenet jord. dk.*

Naverland site



[HOFOR, 2024]

Additional V2-mapped contaminations:

- Herstedostergade 46
- Fabriksparken 26

Drinking water risk:

- Impacts estimated for over 100 years (2)
- cDCE concentrations expected up to 20 µg/L (2)

(2) HOFOR (Nov. 2024). *Naverland 26A-B, Albertslund - Forureningskilder, potentialeforhold og forureningsudbredelse - 2023*. Tech. rep. HOFOR Plan Vand.

Objectives

Aim: Investigate three strategies for cDCE degradation in limestone.

Abiotic	Biotic	Combined
Chemical reduction	Biostimulation Bioaugmentation	Biostimulation Bioaugmentation Chemical reduction

Hypothesis: Combined strategy would perform better than the abiotic and biotic strategies alone.

Agenda

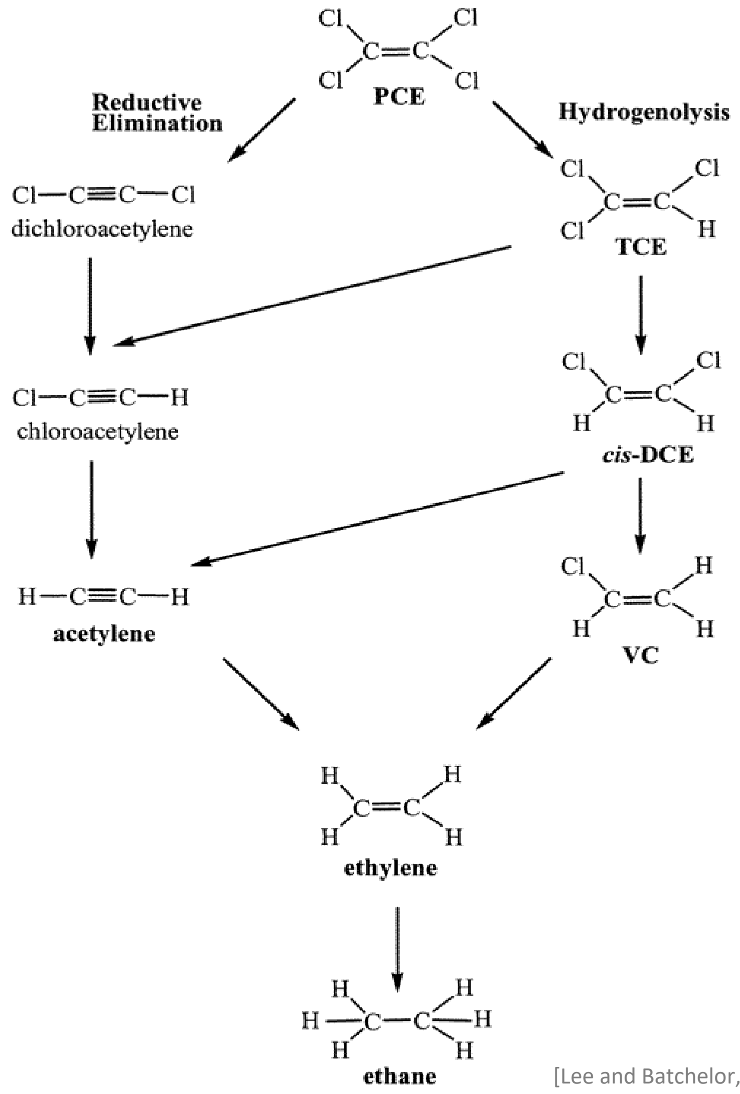
- Background theory
- Methods
- Results
- Discussion
- Conclusion

Chlorinated ethenes

- Dry-cleaning solvents and metal degreasing agents

- Proven carcinogenicity (TCE and VC) (3)
- 1990s: phase-out from European market

- Frequent aquifer pollutants
- PCE and TCE often released as DNAPLs



[Lee and Batchelor, 2002]

(3) ECHA, https://chem.echa.europa.eu/

Reductive dechlorination – a biotic degradation pathway

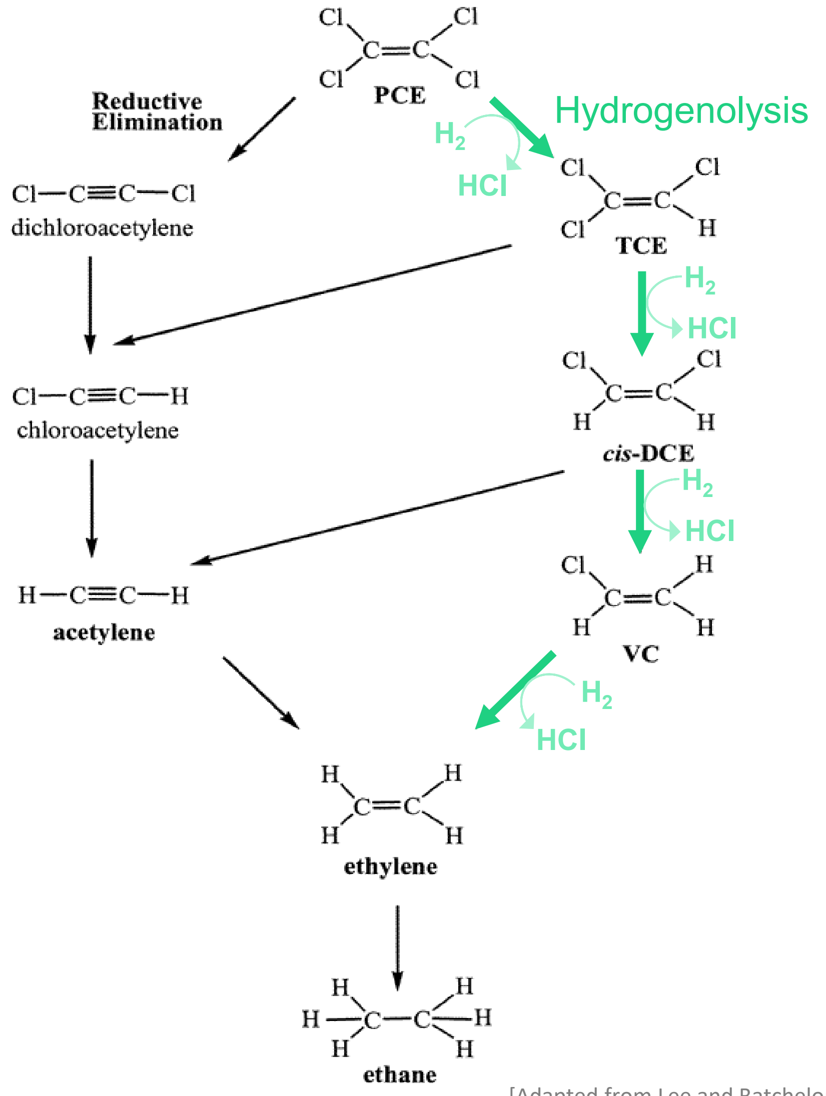
- Hydrogenolysis reaction
- Complete dechlorination: only some *Dehalococcoides* strains

Redox conditions

- Iron-reducing conditions: PCE and TCE to DCE
- Sulfate-reducing conditions: DCE and VC to ethene

Enhanced reductive dechlorination

- Biostimulation: electron donors, nutrients
- Bioaugmentation: bacterial culture



[Adapted from Lee and Batchelor, 2002]

Chemical reduction – an abiotic degradation pathway

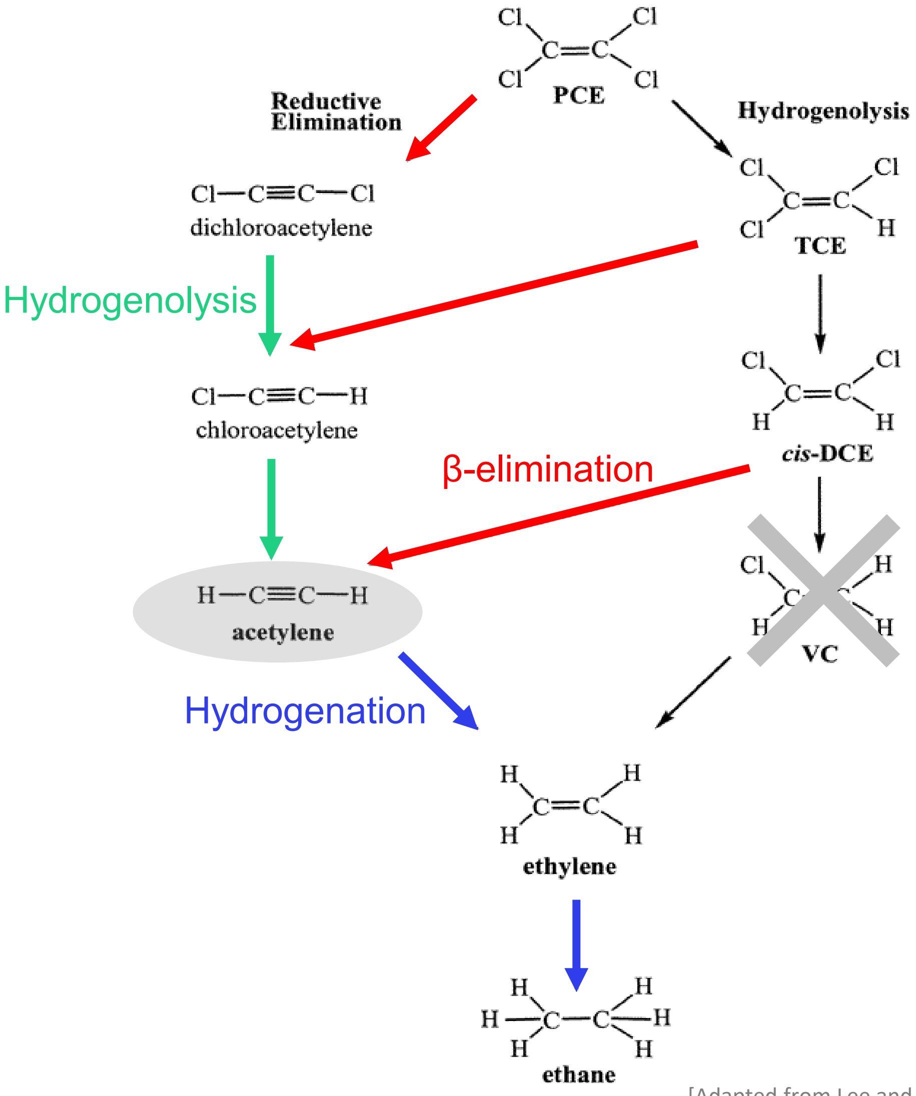
- β-elimination reaction
- Lower production of VC

Direct chemical reaction

- Zero-Valent Iron (ZVI)
- $Fe^0 + RCl + H^+ \rightarrow Fe^{2+} + RH + Cl^-$

Indirect chemical reaction

- Reaction with iron precipitates
- Example: *EHC® Liquid Reagent Mix* by Evonik (Ferrous gluconate $C_{12}H_{22}FeO_{14}$)



[Adapted from Lee and Batchelor, 2002]

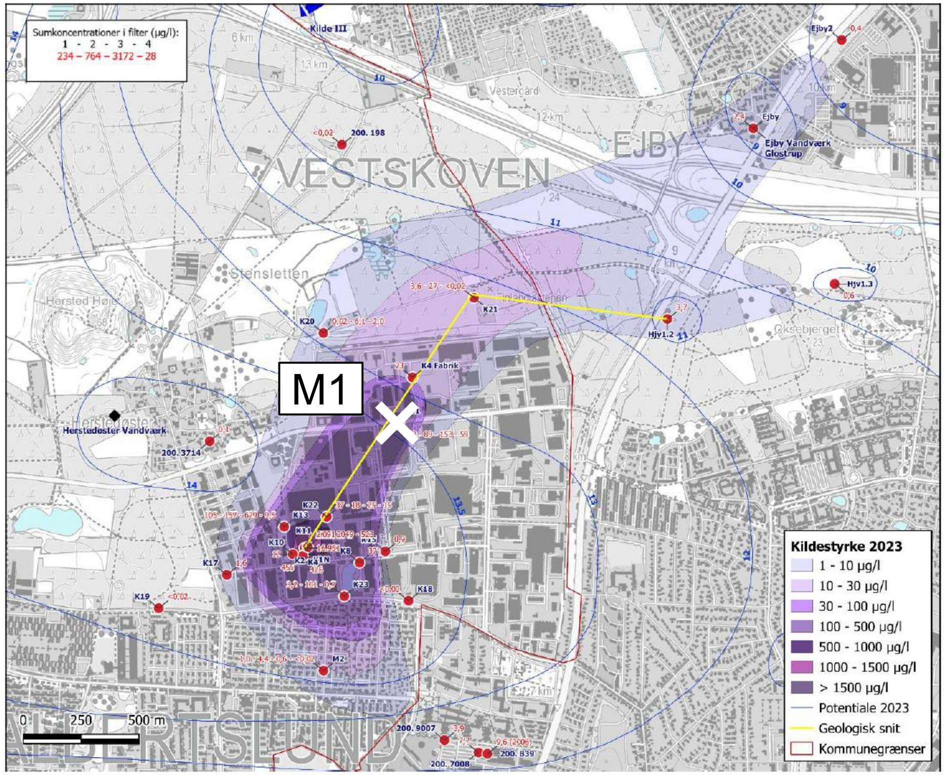
Limestone processing and GW collection

Limestone

Bryozoan type, Faxe Quarry

Groundwater

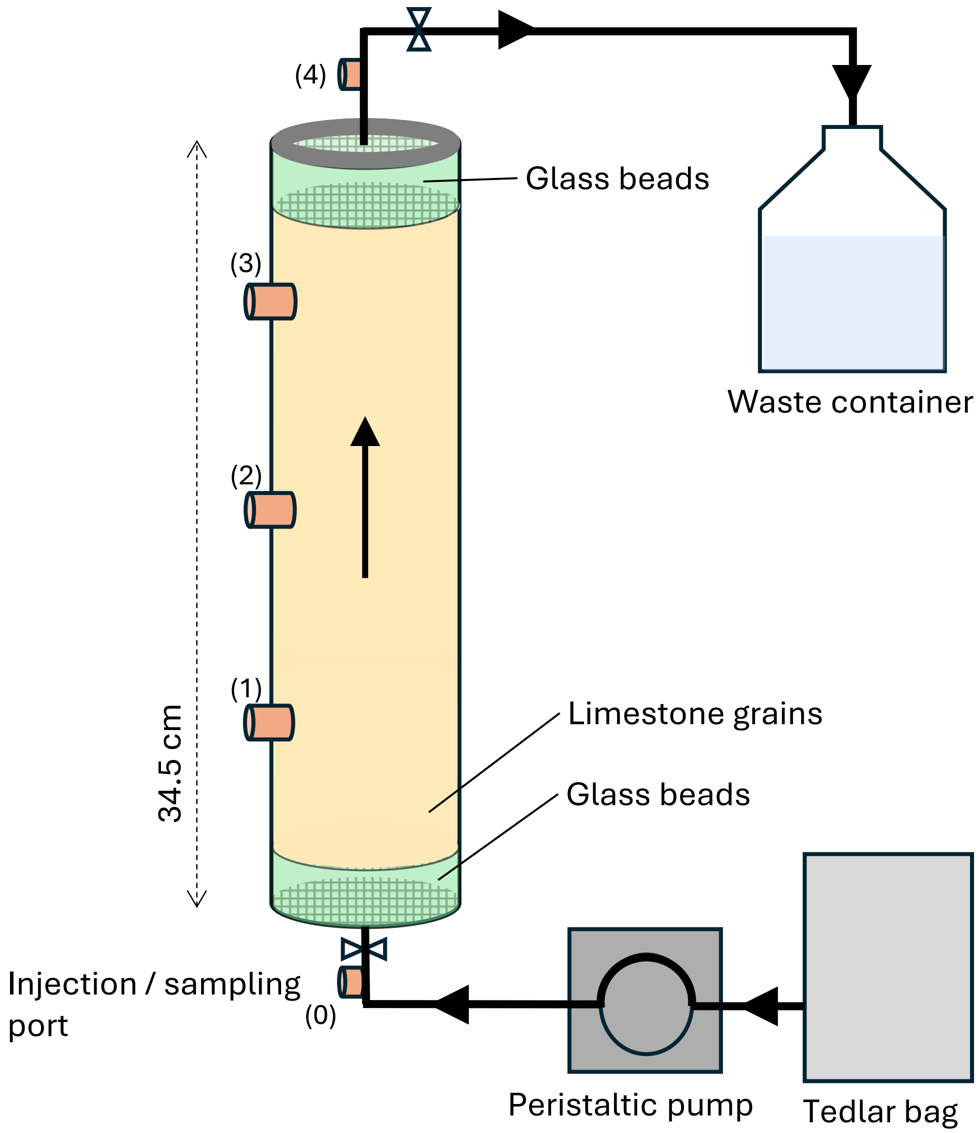
Well M1, 700 m from source



[HOFOR, 2024]

Parameter	Value
pH	6.7
cDCE [µg/L]	184.9
VC [µg/L]	0.4
O_2 [mg/L]	0.02
NO_3^- [mg/L]	2.5 *
Fe^{2+} [mg/L]	2.1
SO_4^{2-} [mg/L]	101.1

Experimental design

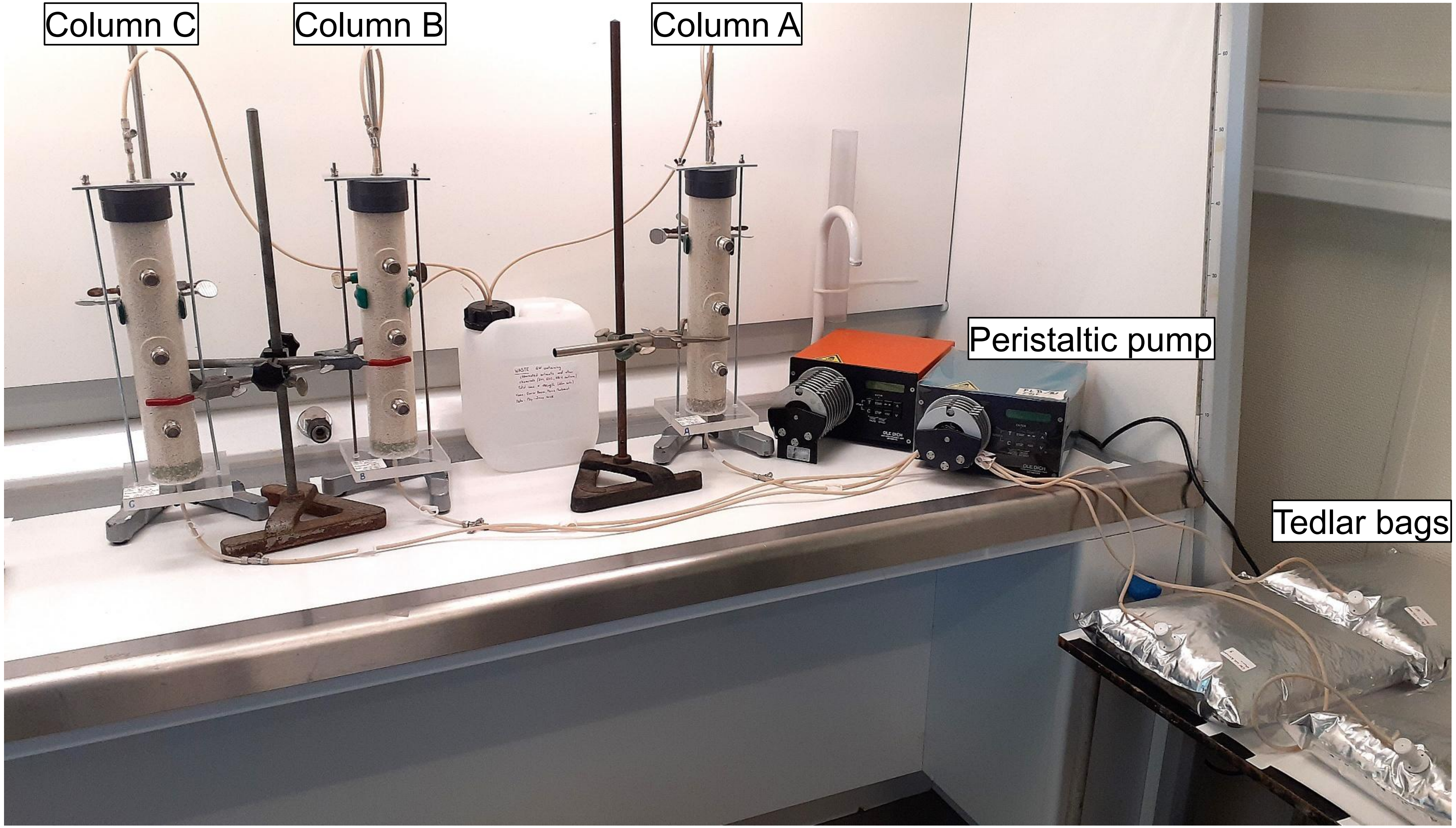


- Replicate saturated aquifer conditions
- Transport processes

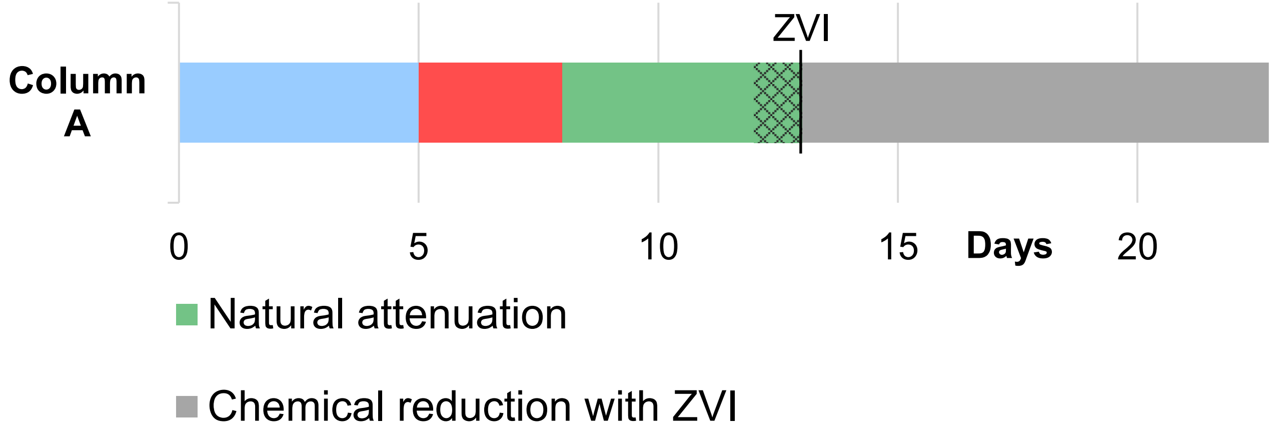
- Limestone grains 1-2 mm ~ proxy fractured limestone
- Dry packing method

- Flow = 0.17 mL/min → Pore velocity = 90 m/yr
- Porosity = 45%
- HRT = 1.3 days
- PV = 320 mL

Experimental set-up



Column A – Abiotic



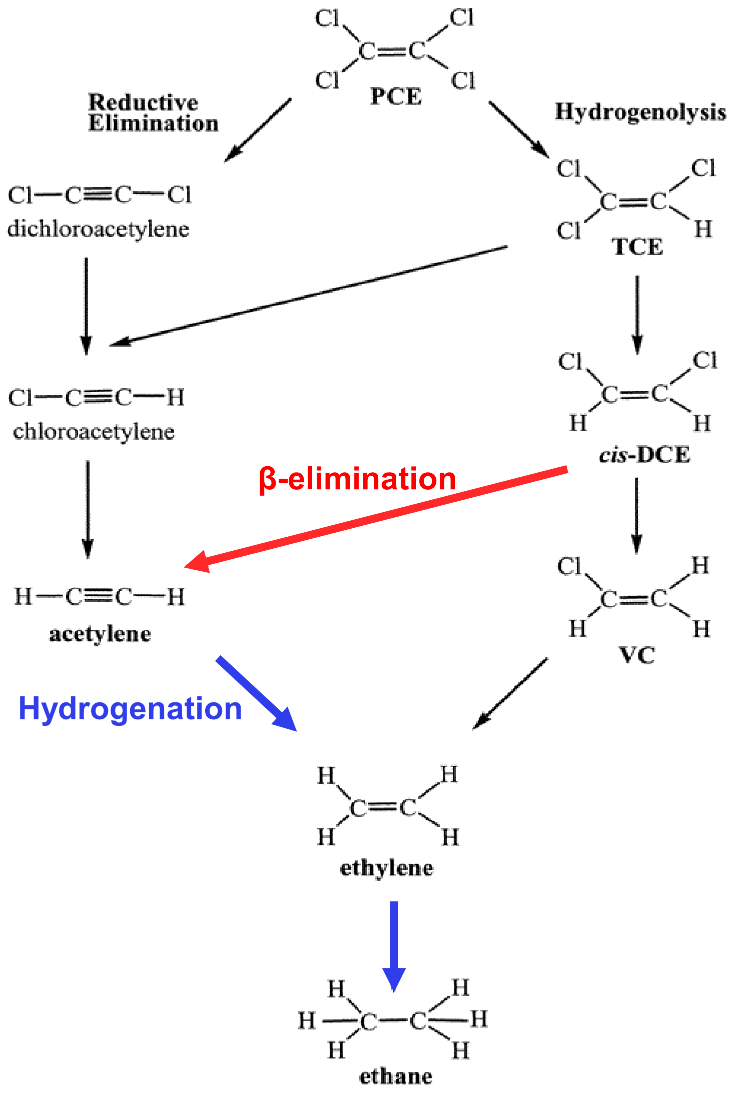
Chemical reduction

SmZVI by Nanoiron:

- Colloidal suspension, Microscale (~ 10 µm) & Sulfidated

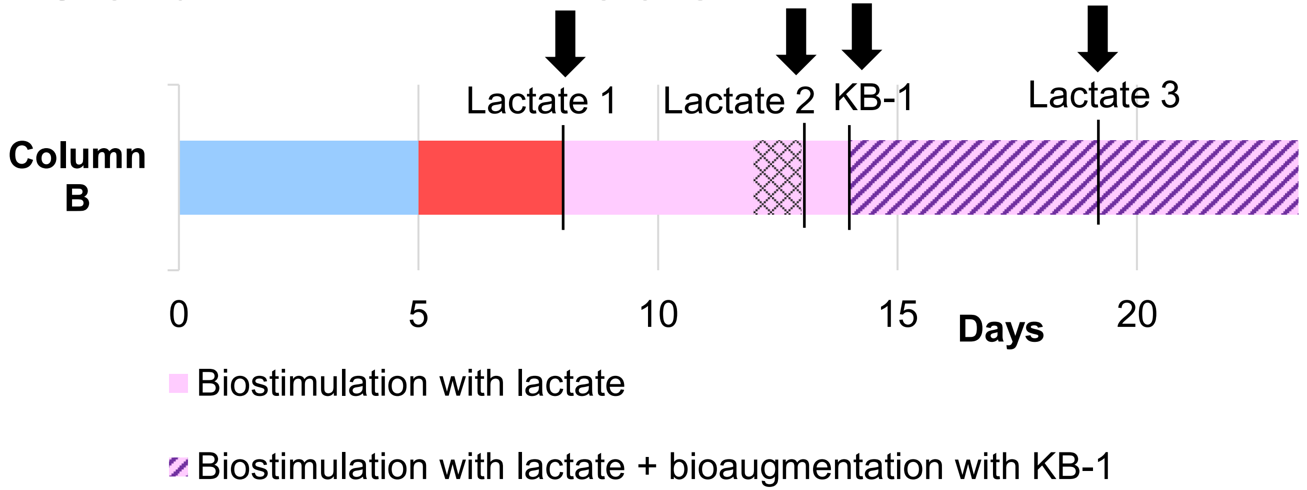
Injections through lateral ports:

- 11 gFe0/kg limestone ~ 1.1 % by weight
- 2 gFe0/L GW for 15 PV



[Adapted from Lee and Batchelor, 2002]

Column B – Biotic



Biostimulation

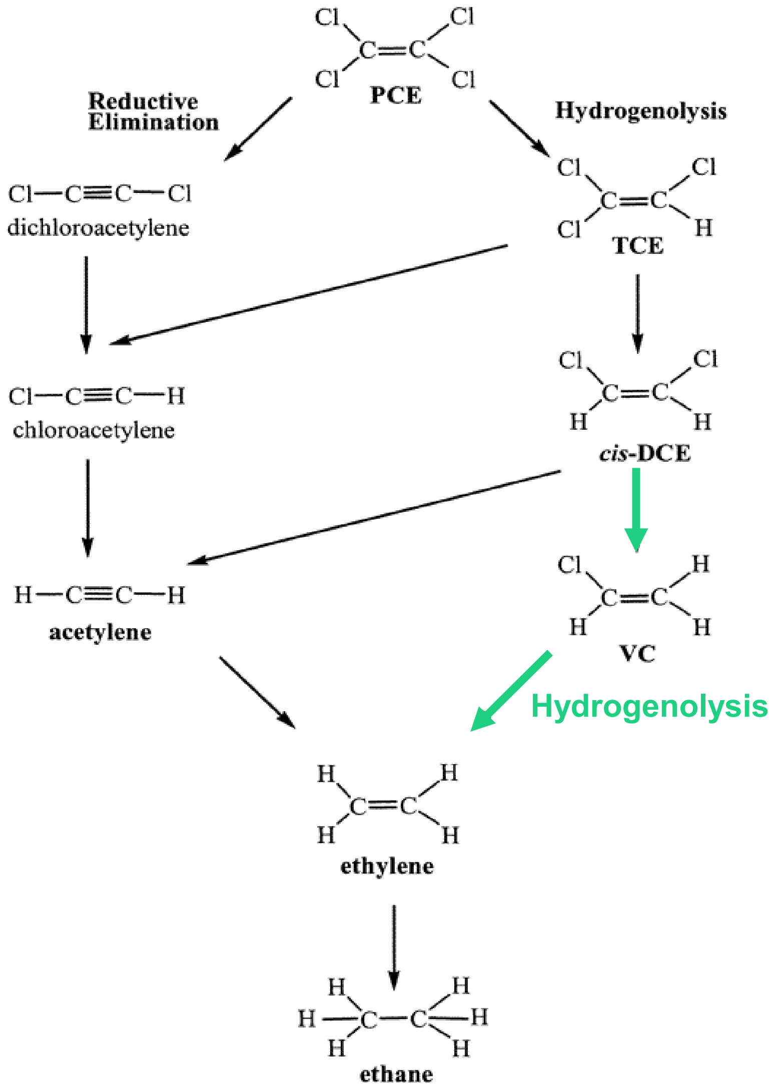
60% sodium lactate solution

- Electron donor
- Immediately bioavailable
- 3 injections of 0.52 mL ~ 320 mg lactate each

Bioaugmentation

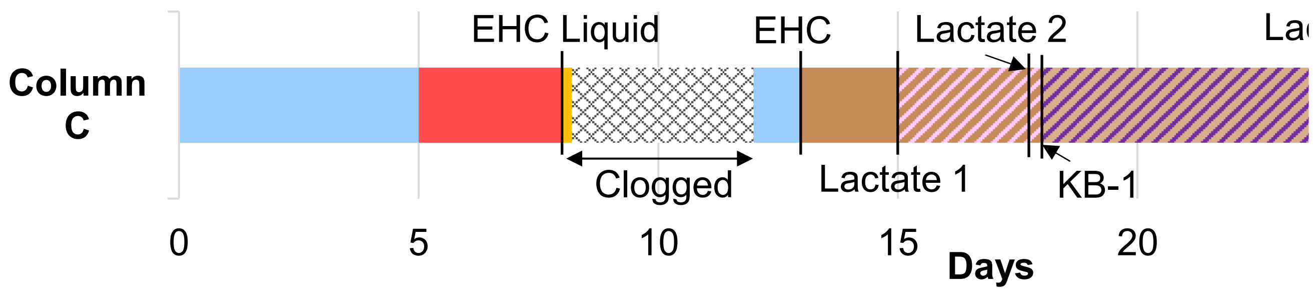
KB-1®

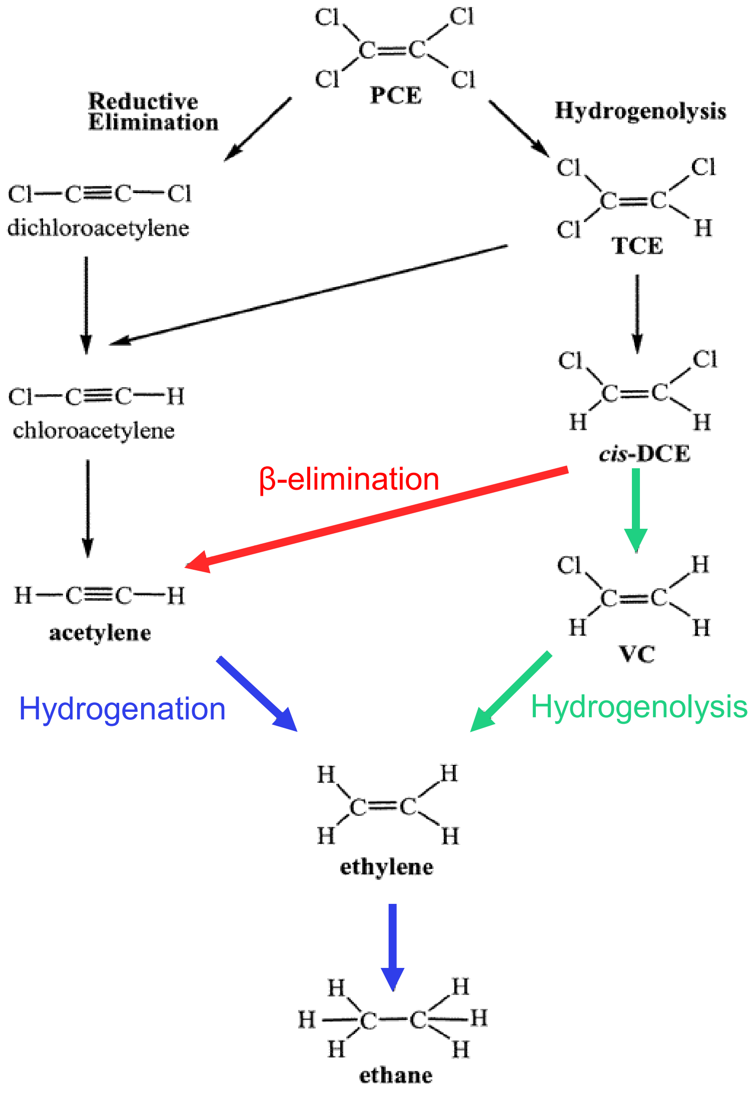
- Contains *Dehalococcoides*
- 1E+9 cells/L GW
- Injection of 3.5 mL



[Adapted from Lee and Batchelor, 2002]

Column C – Combined (abiotic + biotic)





[Adapted from Lee and Batchelor, 2002]

Chemical reduction

EHC® Liquid Reagent Mix alone

- 33 g ferrous gluconate $C_{12}H_{22}FeO_{14}$ ~ 4.1 g iron
- 150 mL solution injected (~ 0.43 PV)

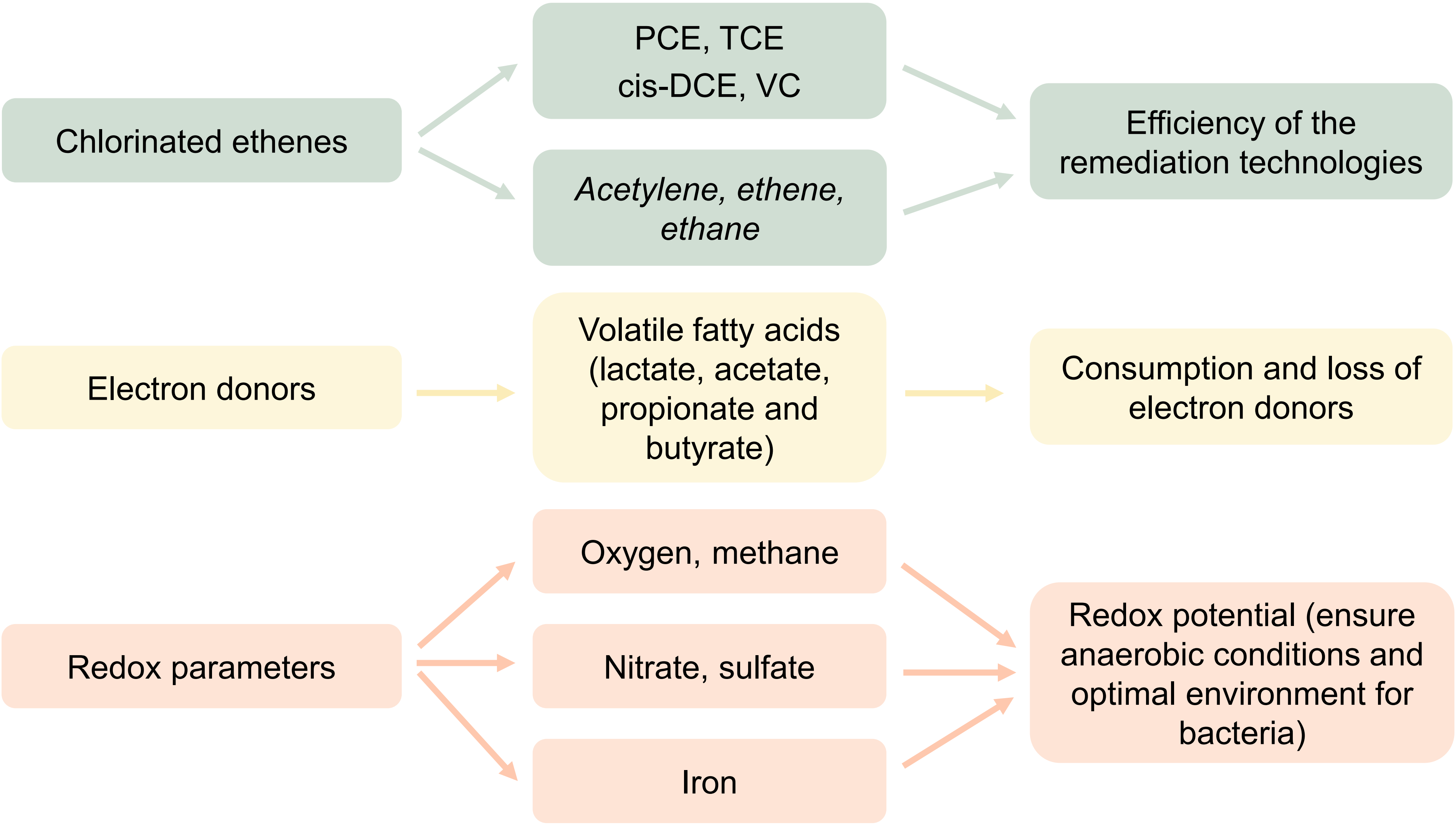
Biostimu

60% sodium lac

- Compensate f
- 3 injections of

320 mg lactate ea

Chemical analyses



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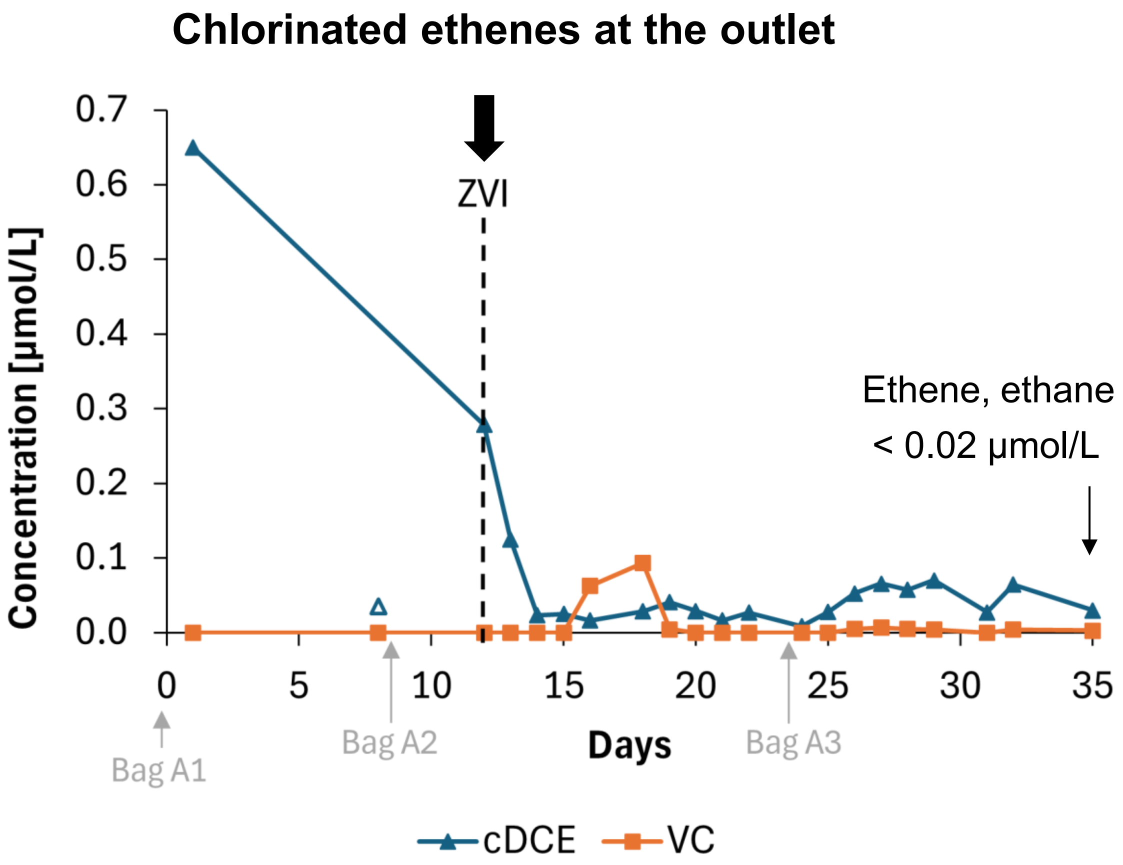
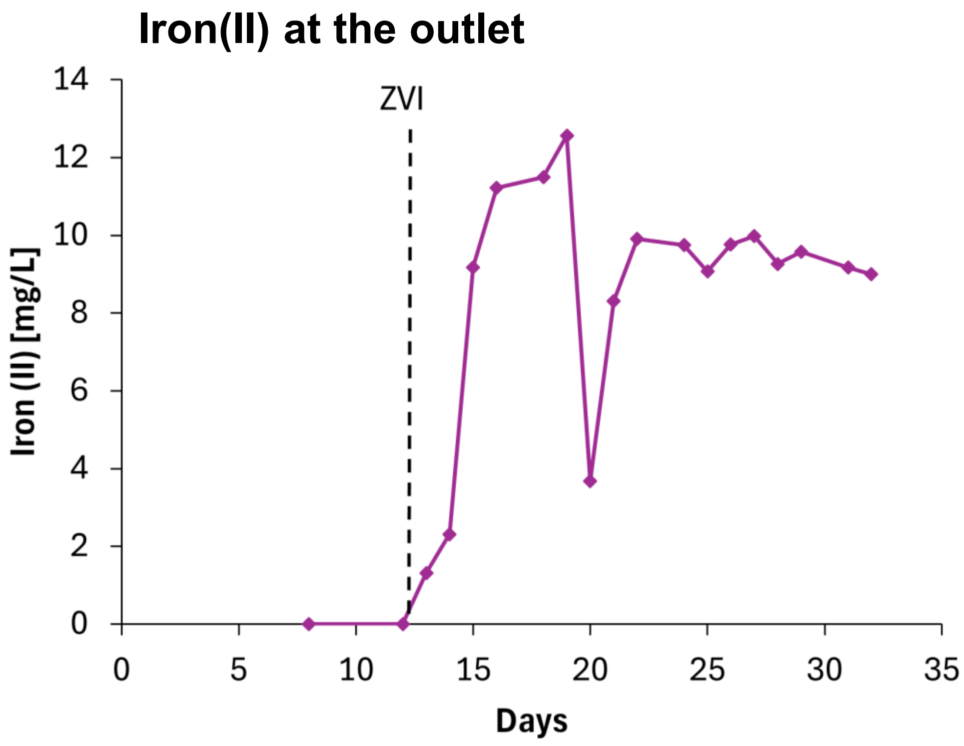
Column A – Abiotic





- ➢ Corrosion of ZVI particles

- ➢ Deficit in molar balance
- ➢ Assumption ethene oxidation to CO2

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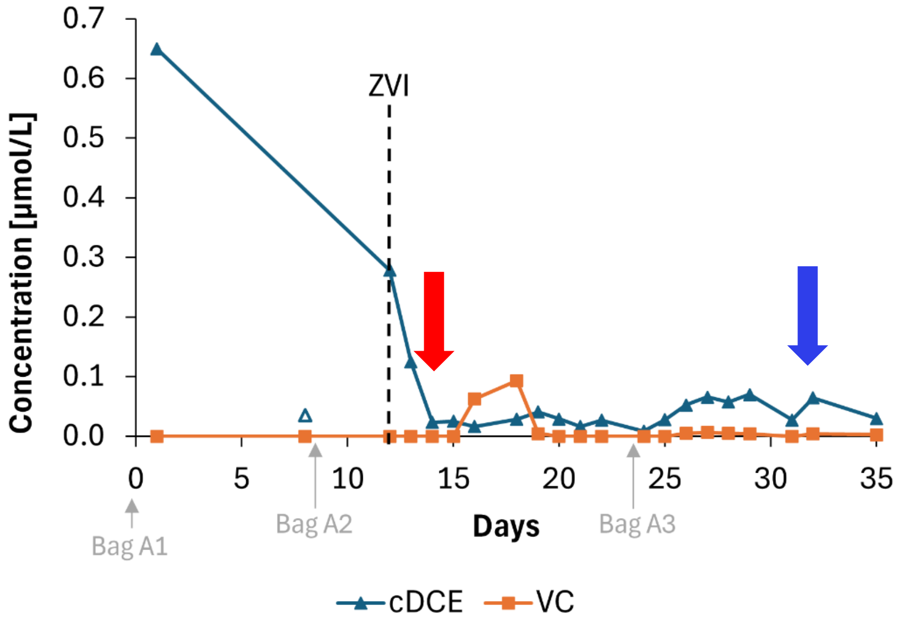
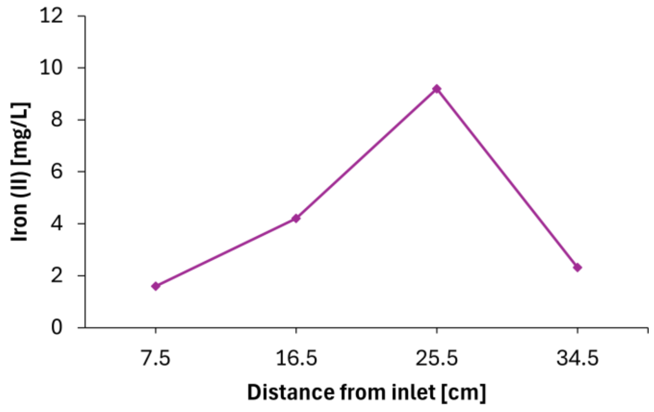
Column A – Abiotic

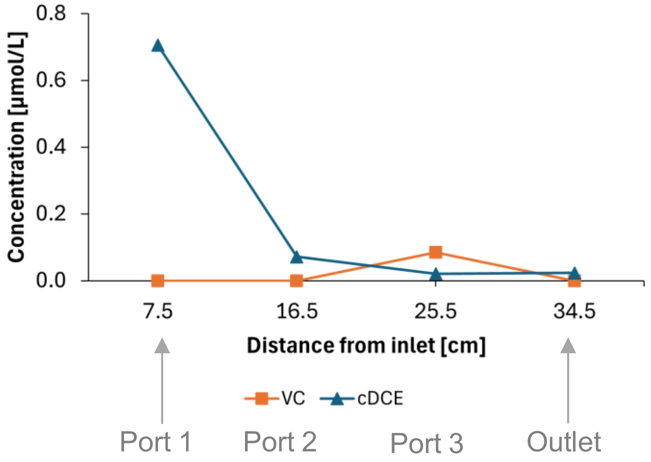
Chlorinated ethenes at the outlet



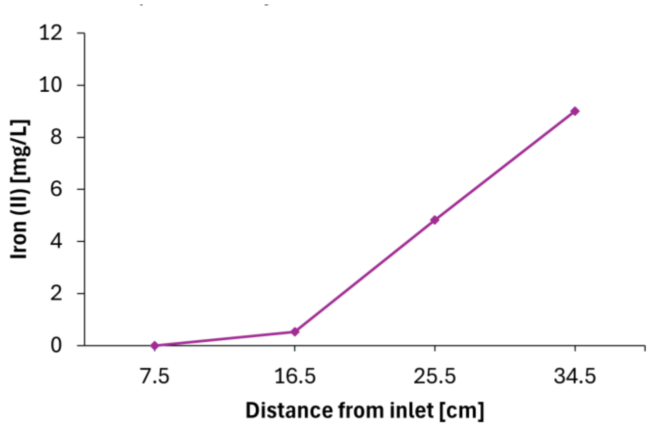
Profiles CE and iron (II)

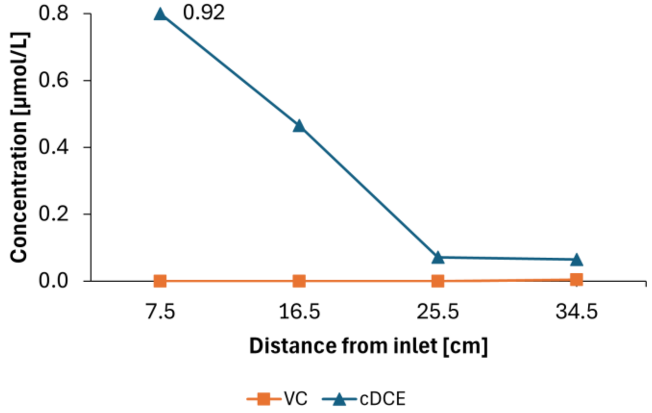
Day 14





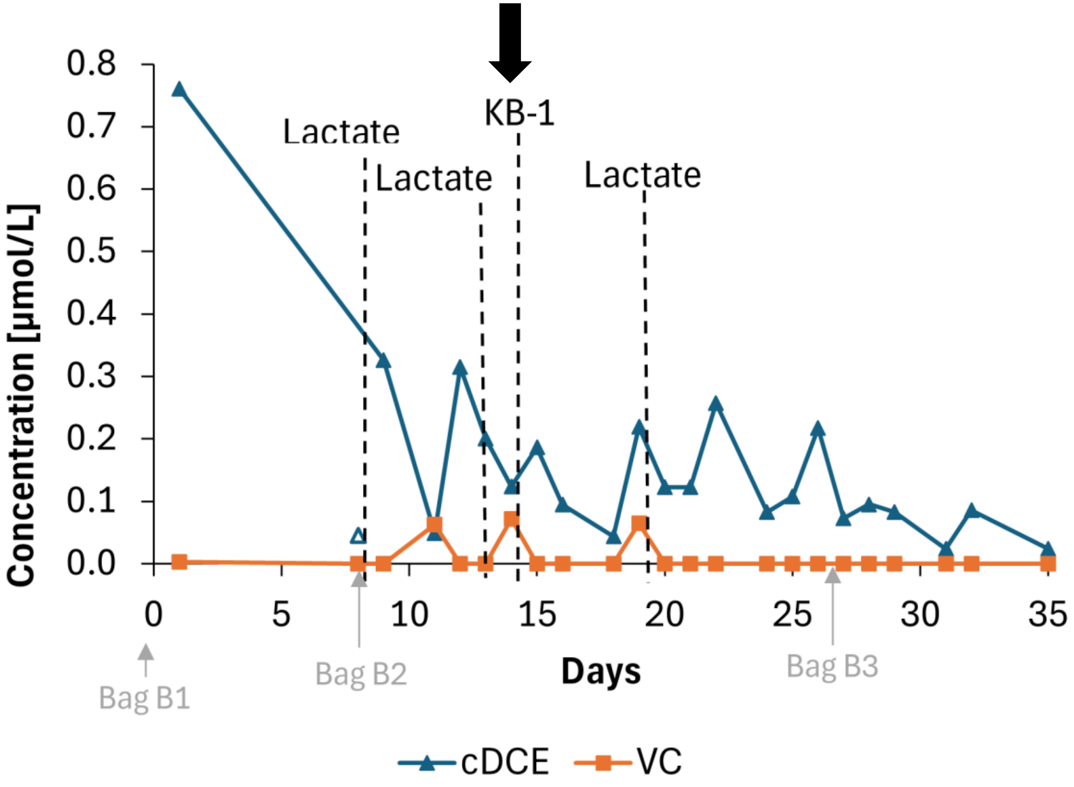
Day 32



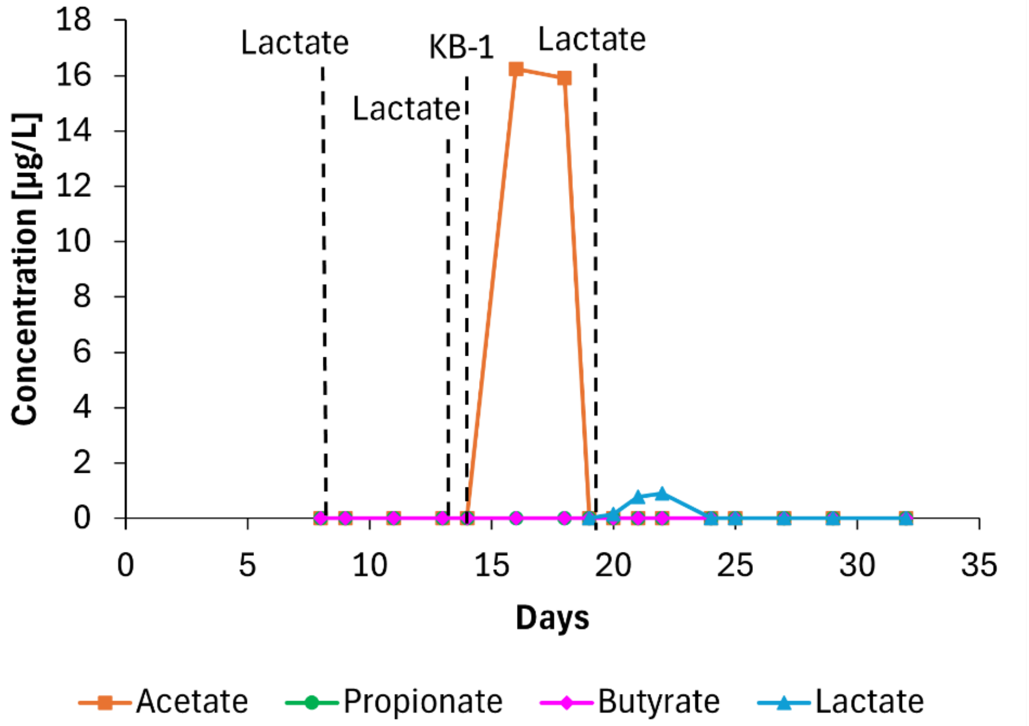


Column B – Biotic

Chlorinated ethenes at the outlet



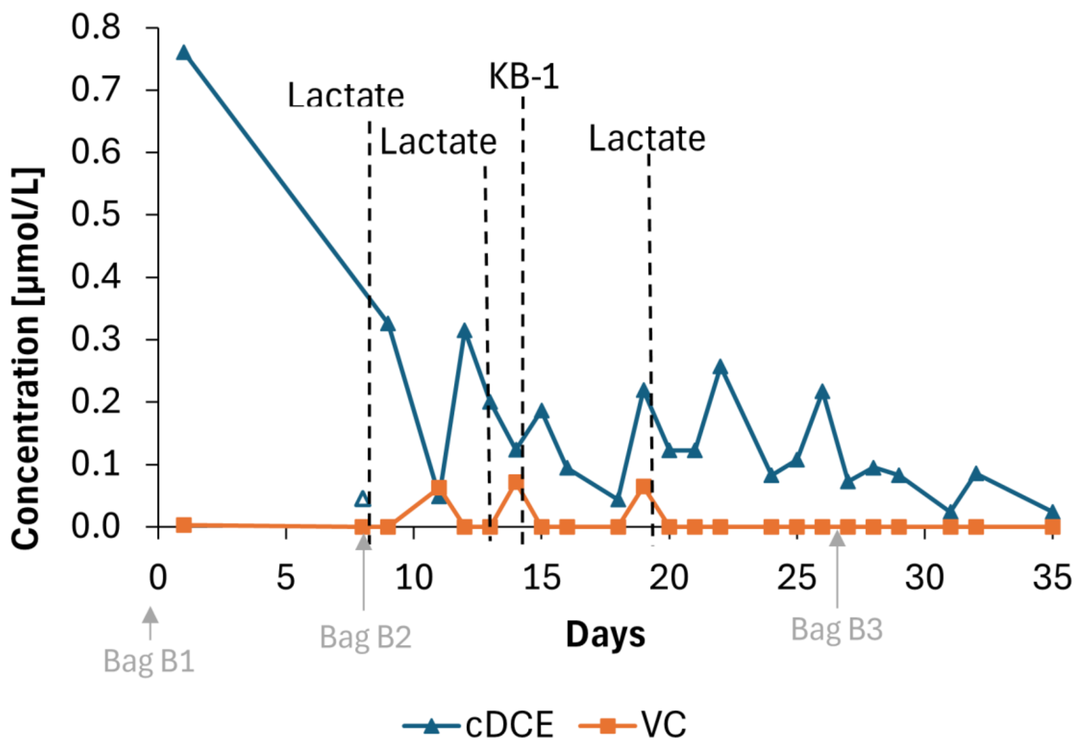
VFAs at the outlet



- Microbial activity

Column B – Biotic

Chlorinated ethenes at the outlet



After lactate injections and before KB-1® injection:

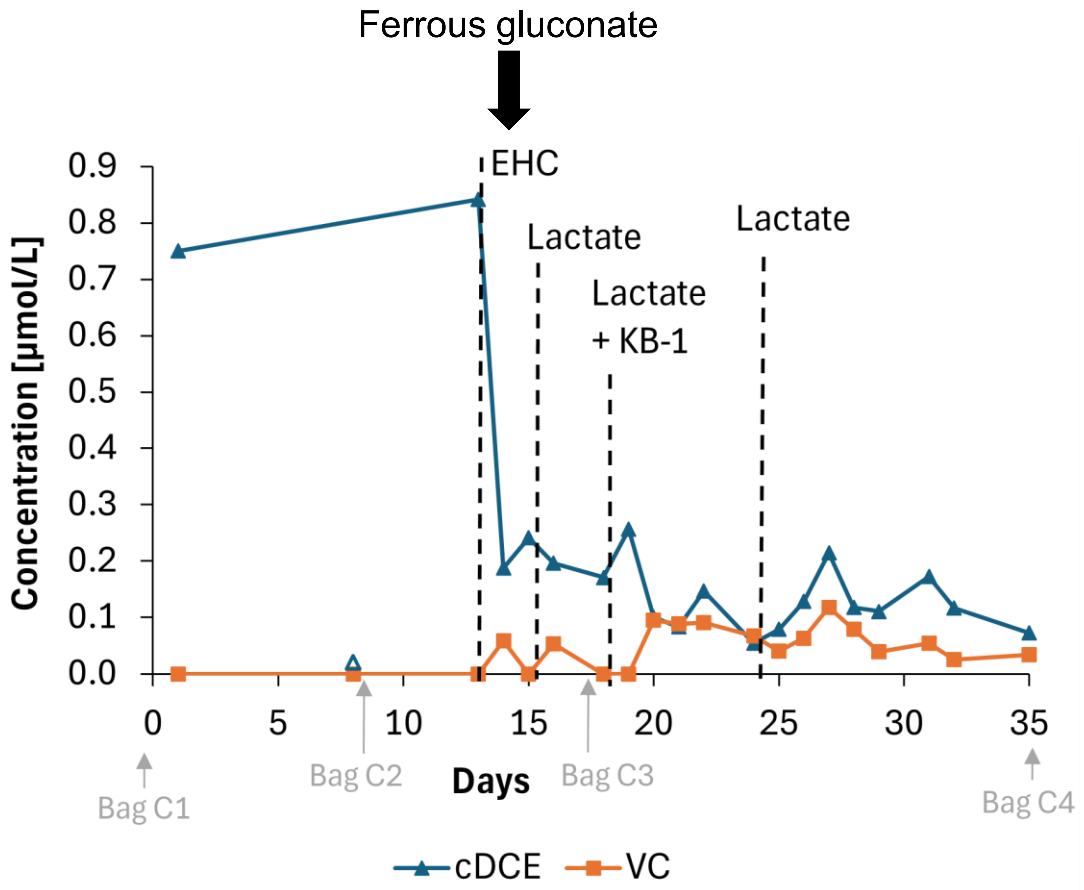
- Decreasing cDCE concentrations
- Detected VC

- Potential presence of cDCE and VC degrading bacteria in groundwater

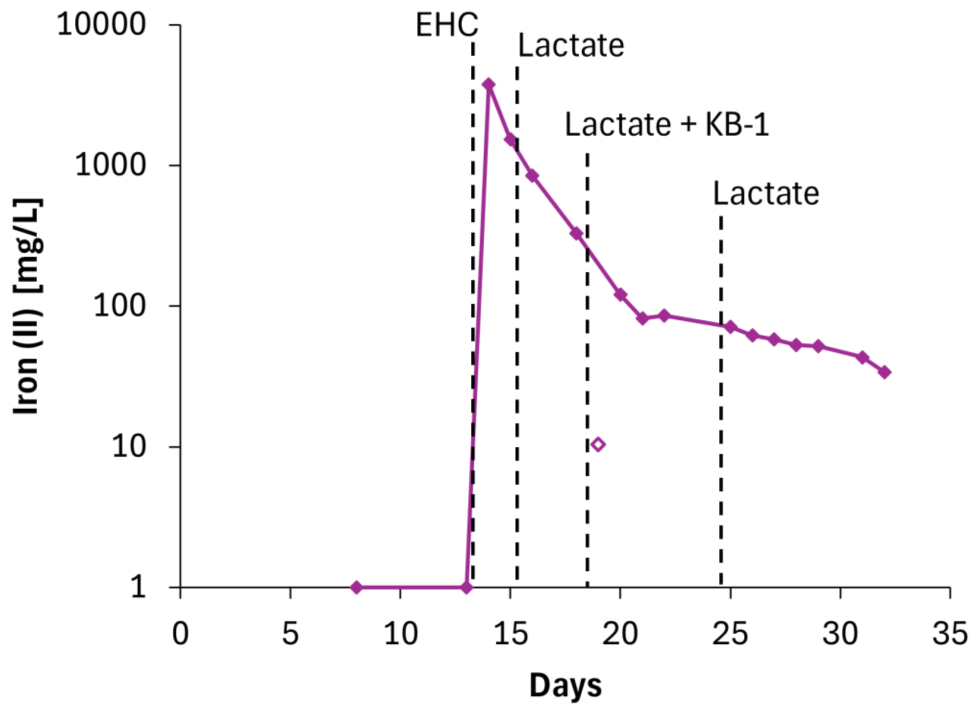
- KB-1® enhances biodegradation

Column C – Combined (abiotic + biotic)

Chlorinated ethenes at the outlet



Iron (II) at the outlet



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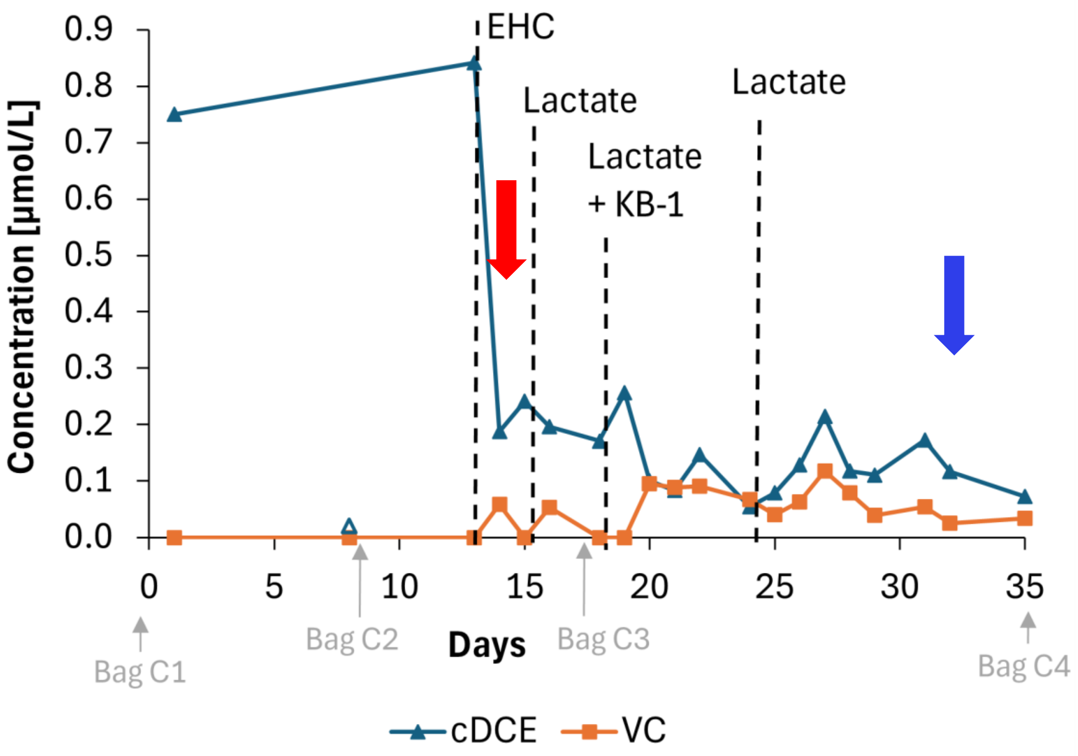
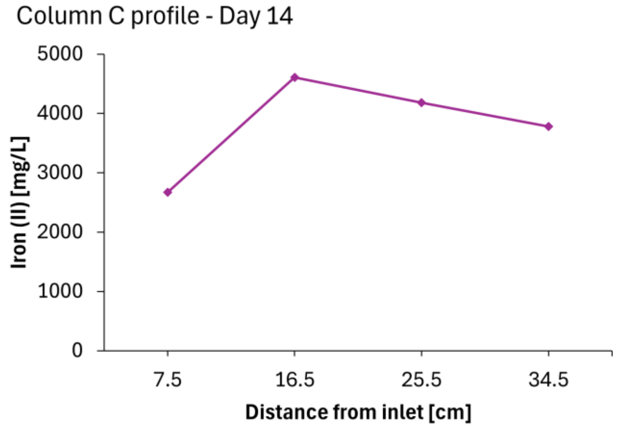
Column C – Combined

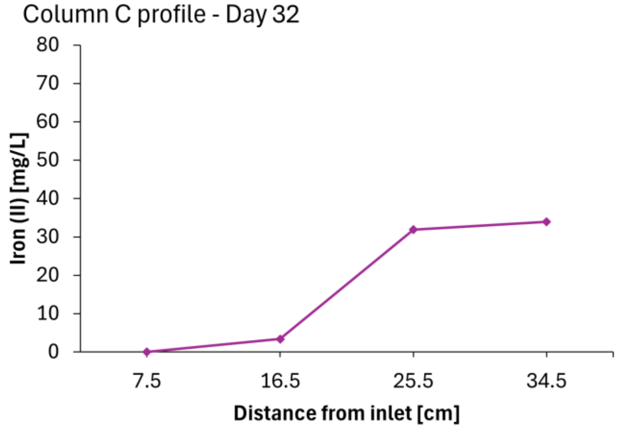
Profiles iron (II) and CE

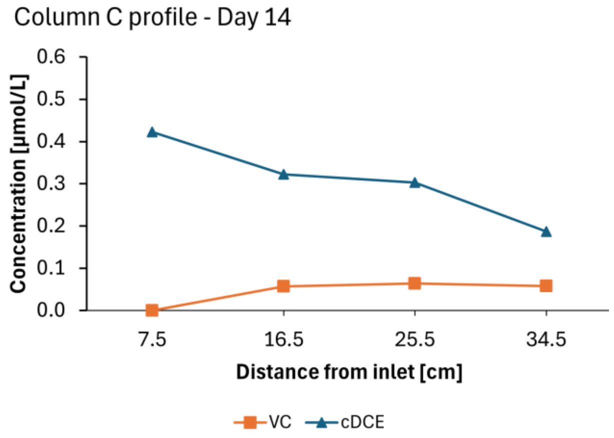
Day 14 Day 32

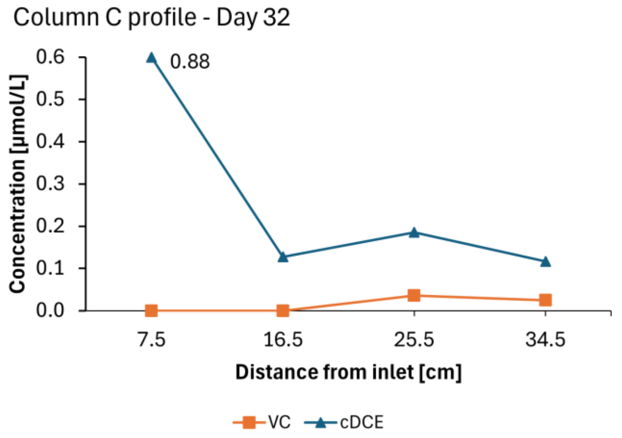
Chlorinated ethenes at the outlet



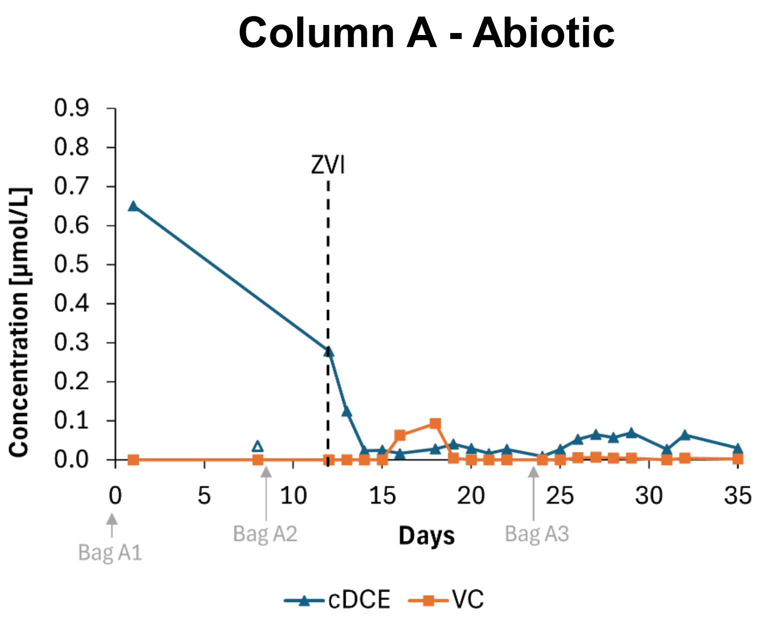


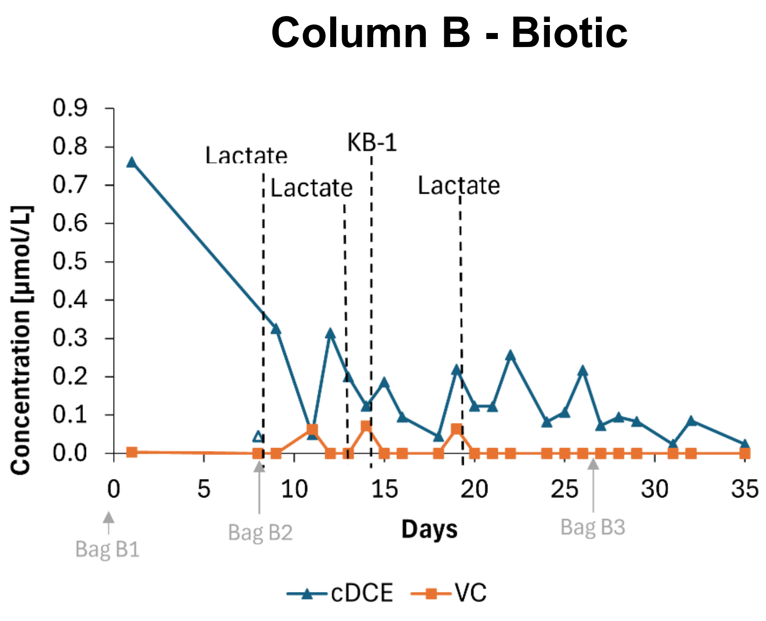


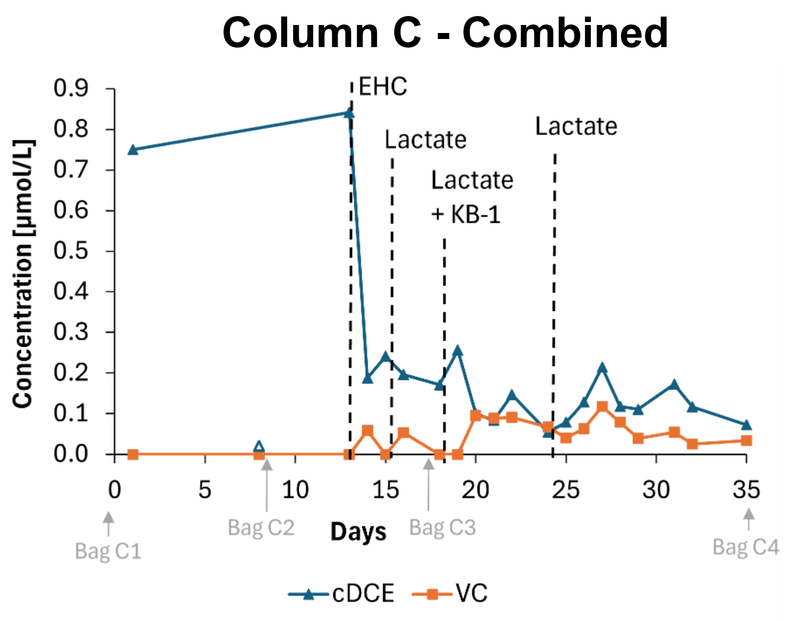




Column comparison and performance







- Low cDCE and almost no VC production
- No VC production after day 19
- VC aways detected from day 20

Ranking performance: Abiotic column > Biotic column > Combined column

Evaluation of the amendments

SmZVI

- Fast and maintained reactivity
- Good longevity (~ 150 days)
- Low mobility

Lactate

- High reactivity
- Almost complete consumption
- High mobility

EHC® Reagent Liquid Mix

- Not conventional use
- Limited longevity
- Formation of iron precipitates
- High mobility

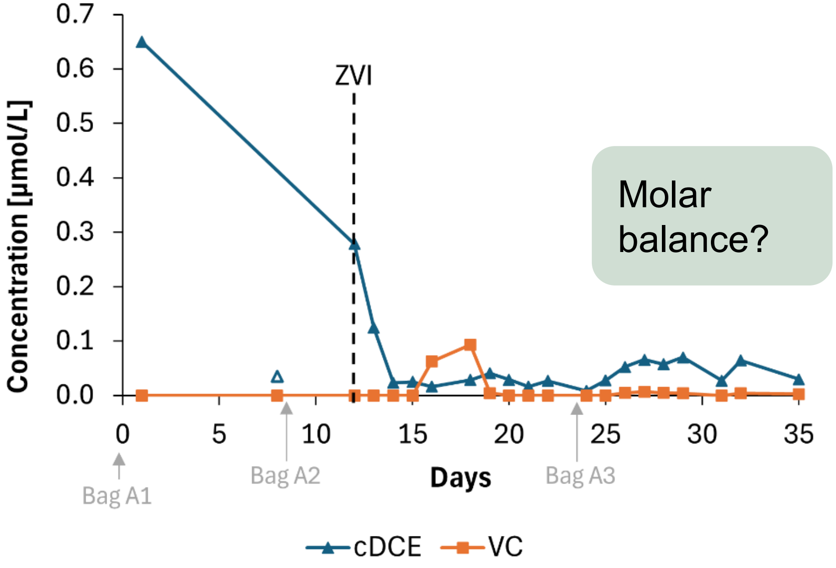
KB-1®

- Longevity determined by conditions
- Limited mobility

Improvement suggestions

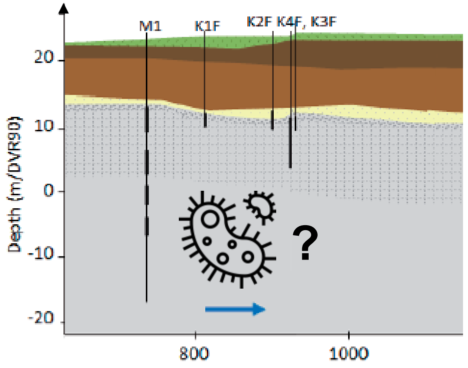
Improve knowledge on degradation

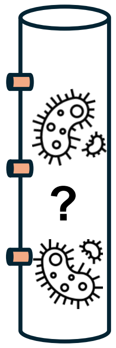
- Measure all degradation products
- Include bacterial analyses



Increase duration of the experiment

- Might improve biotic system performance
- Improve evaluation of longevity



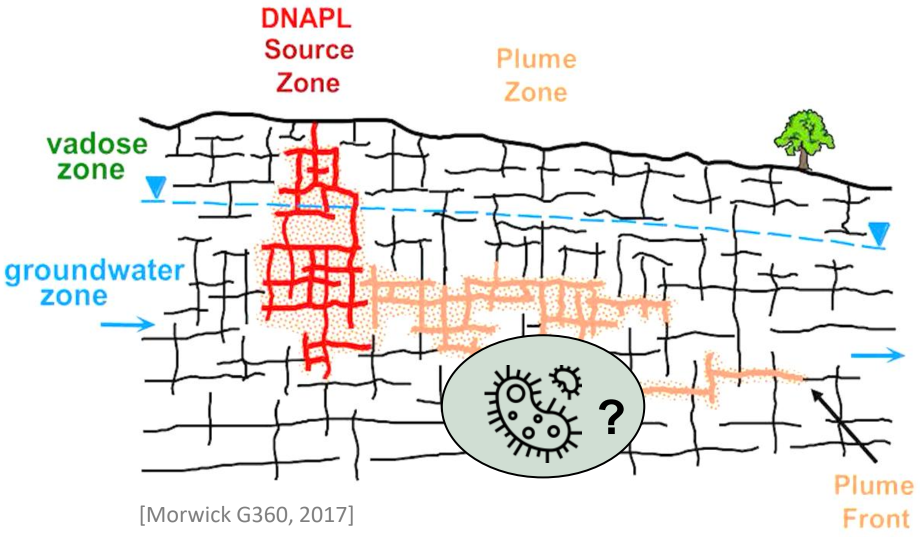


[Modified from Hemdorff, 2013]

Potential for field applications

Challenges

- ➢ Matrix → low permeability, high porosity
- ➢ Contact time between amendments and contaminant

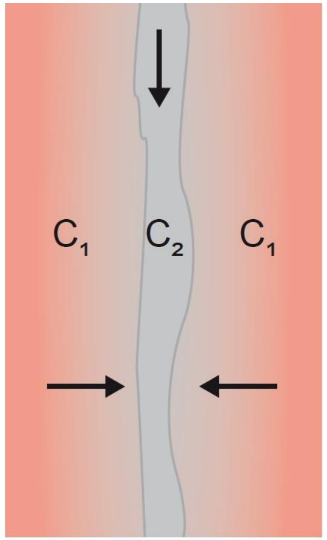


[Morwick G360, 2017]

Amendments → Good mobility and longevity

- ➢ Nano sulfidated ZVI
- ➢ Nano Sulfidated ZVI + lactate (+ KB-1®) [(4)]
- ➢ EHC® Liquid (+KB-1®) [(5)]

Back diffusion
→ Matrix = long-term secondary source



[Bjerg et al., 2017]

(4) Lacinová, Lenka, Martina Černíková, Jaroslav Hrabal, and Miroslav Černík (Oct. 2013). "In-Situ Combination of Bio and Abio Remediation of Chlorinated Ethenes", *Ecological Chemistry and Engineering* S 20.3, pp. 463–473. doi:10 . 2478 / eces - 2013 - 0034.

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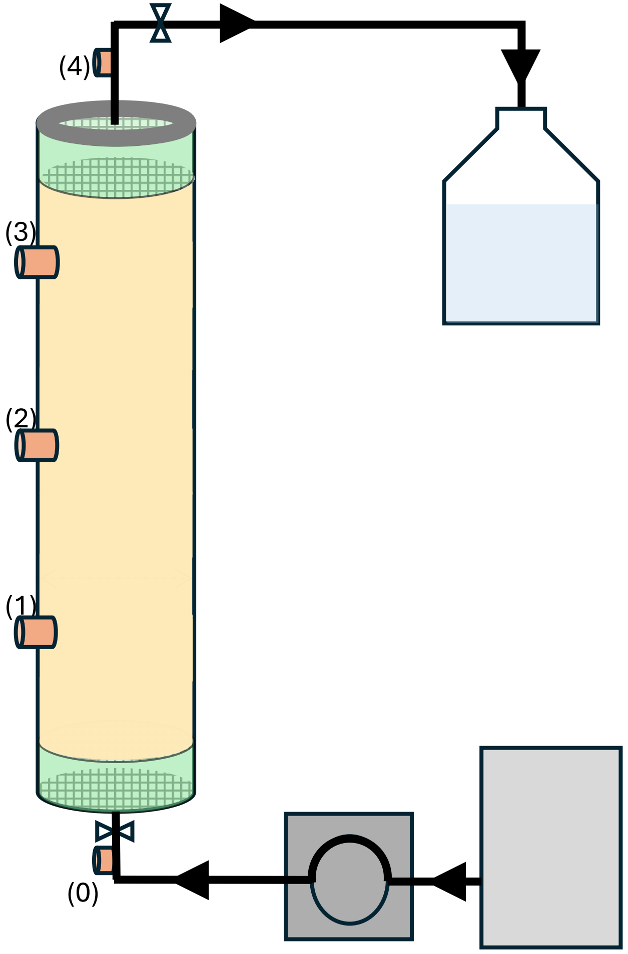
Conclusions

Successful design of continuous-flow column experiment

Initial assessment of mobility and longevity of amendments

Good reactivity of amendments with cDCE

Efficiency columns: abiotic > biotic > combined



Thank you

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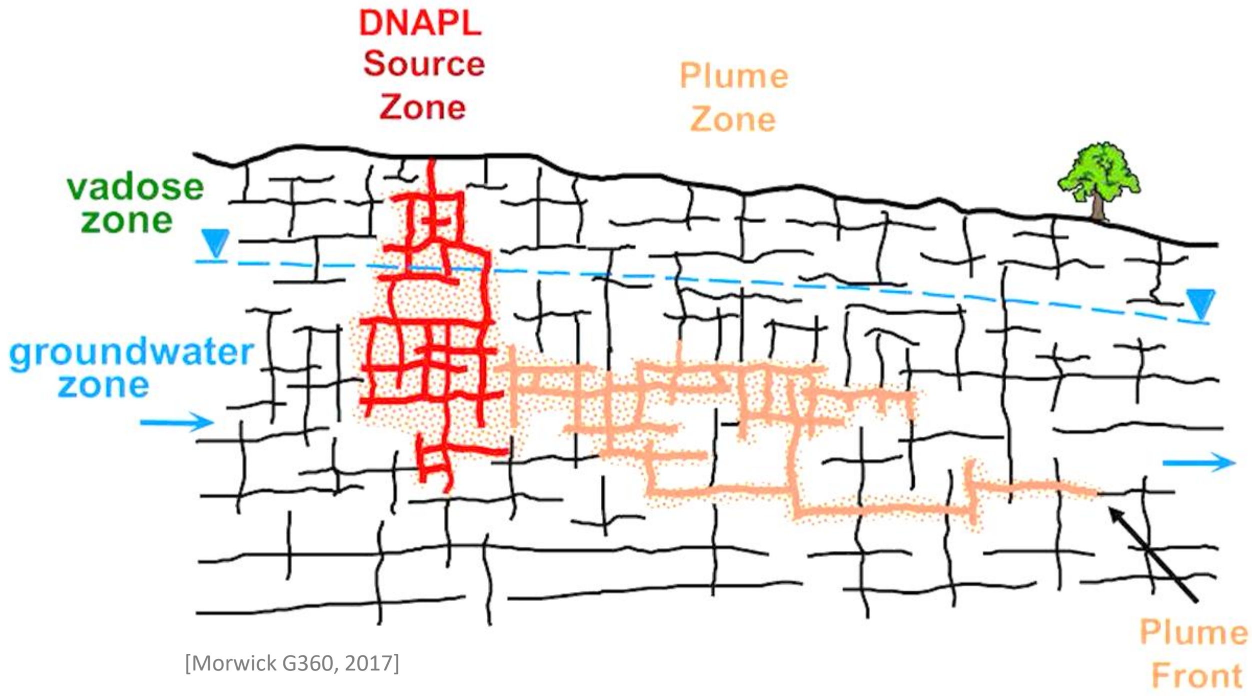
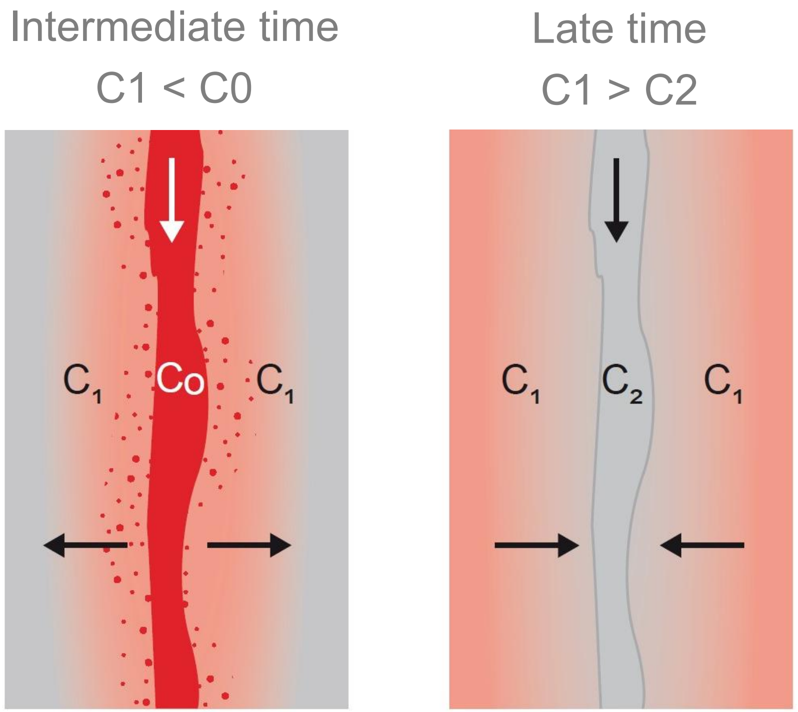
Additional slides

Contaminant transport in fractured limestone aquifers

- Dual porosity medium — matrix vs fractures
- Preferential pathways along the fractures
- Back diffusion
- Matrix as a contamination reservoir

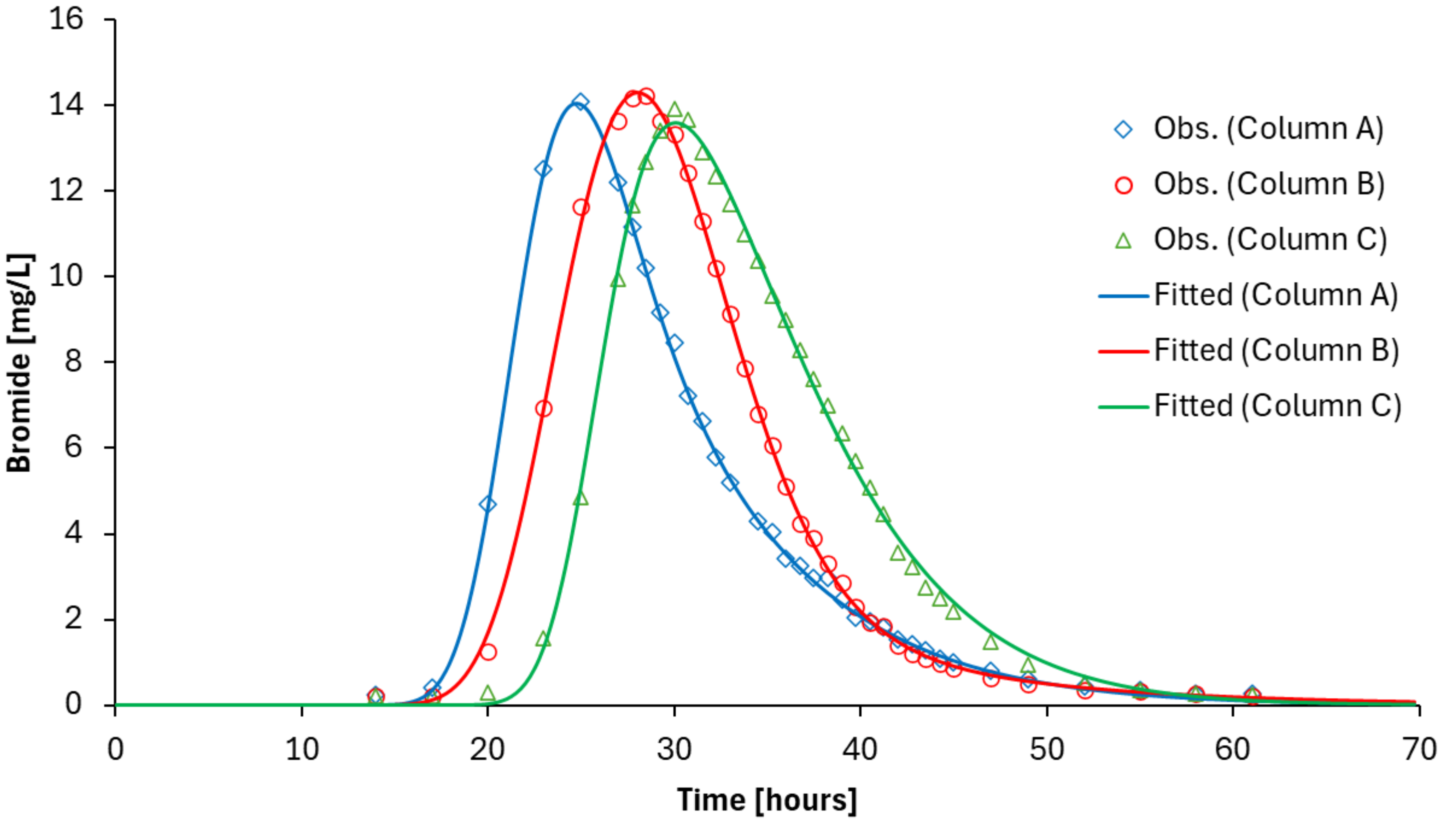


[Morwick G360, 2017]



[Bjerg et al., 2017]

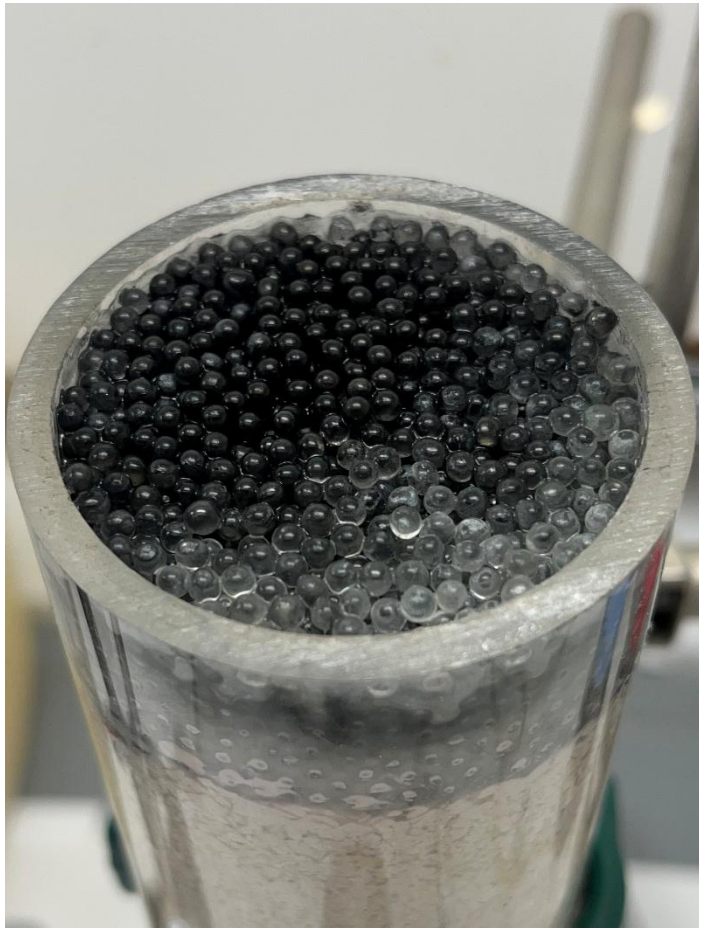
Tracer test



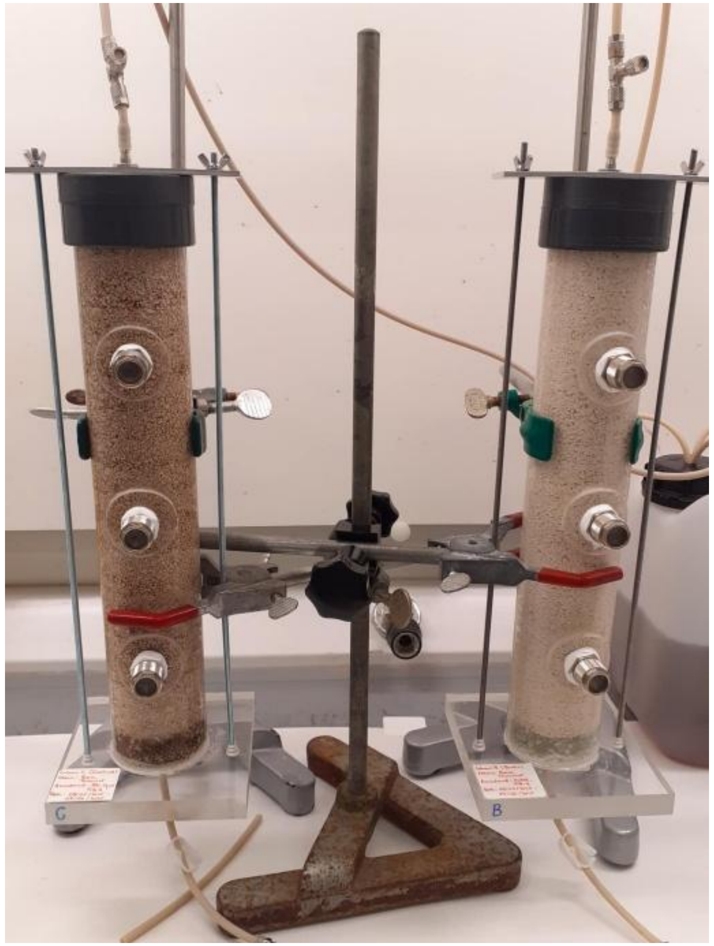
- Two-region non-equilibrium CXTFIT model
- Immobile and mobile regions

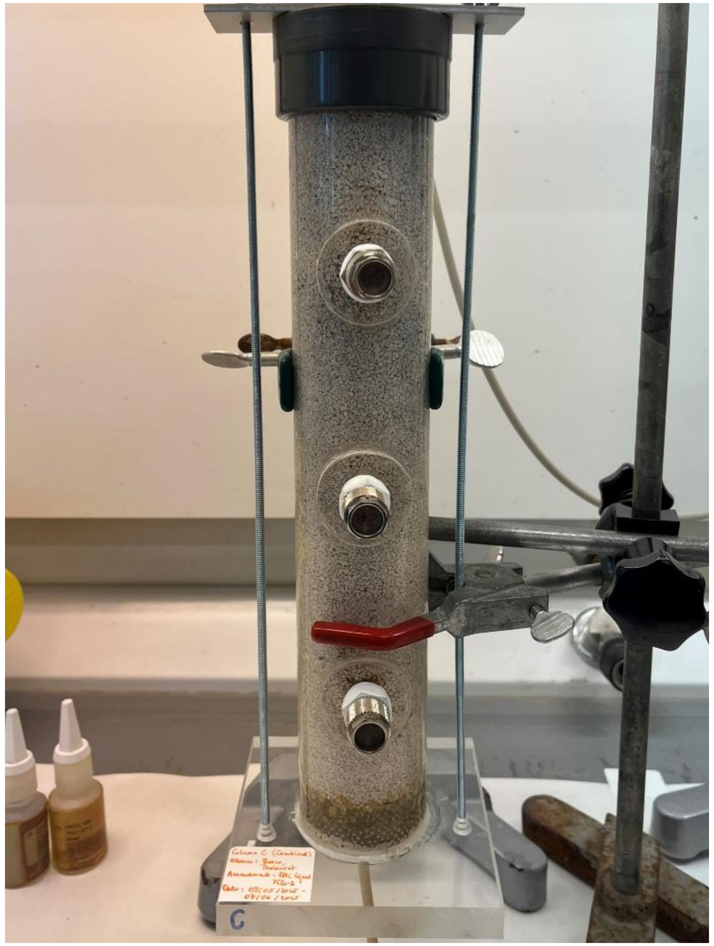
- Effective porosity [42% − 49%]
- Pore volume [295 – 345 mL]
- HRT [1.2 − 1.4 days]

Physical observations – Column A

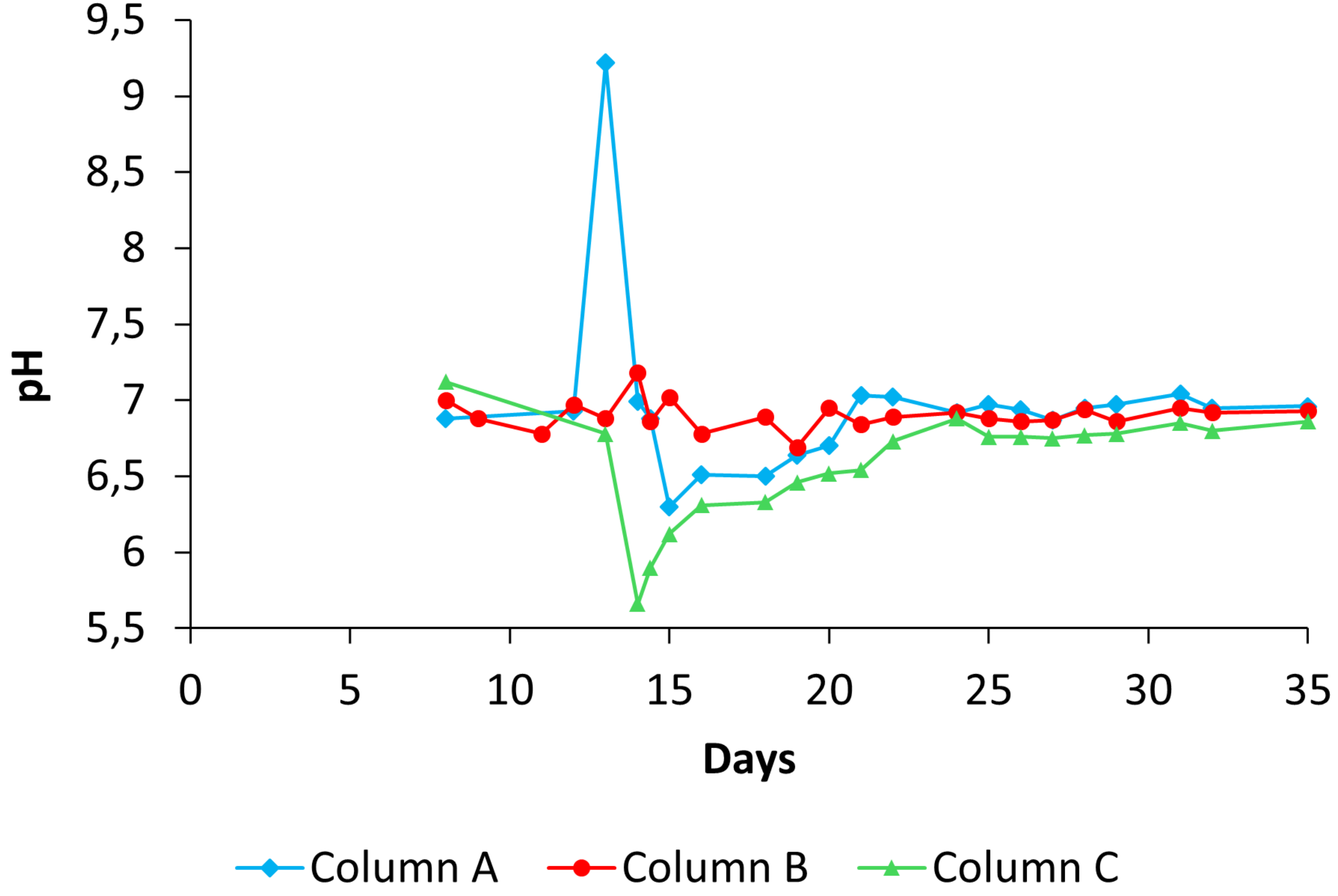


Physical observations – Column C





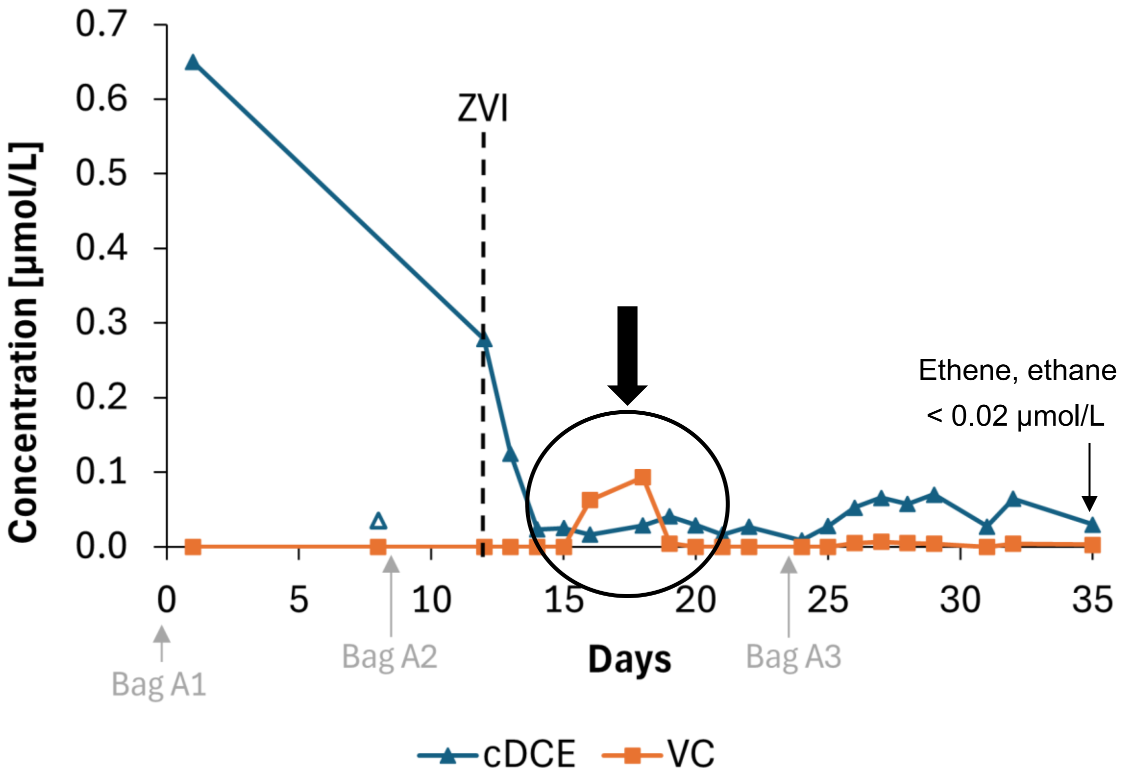
pH



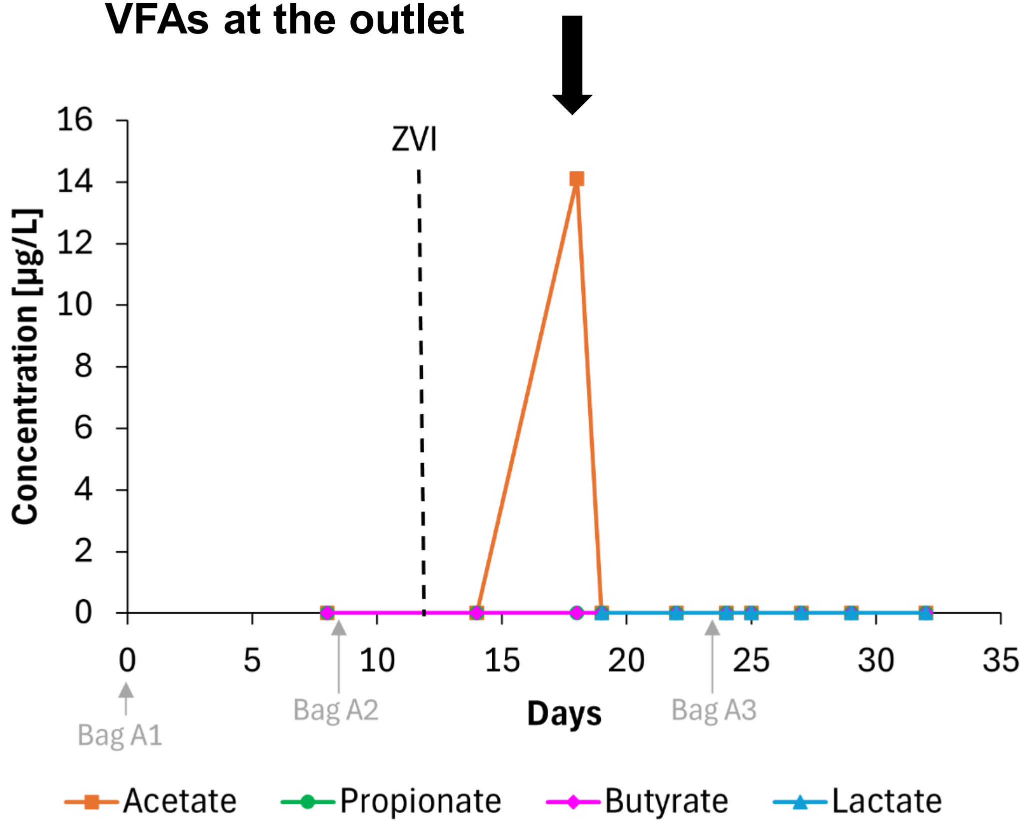
9,5
9
8,5
8
7,5
7
6,5
6
5,5
pH
0
5
10
15
20
25
30
35
Days
Column A
Column B
Column C

Column A - Abiotic

Chlorinated ethenes at the outlet



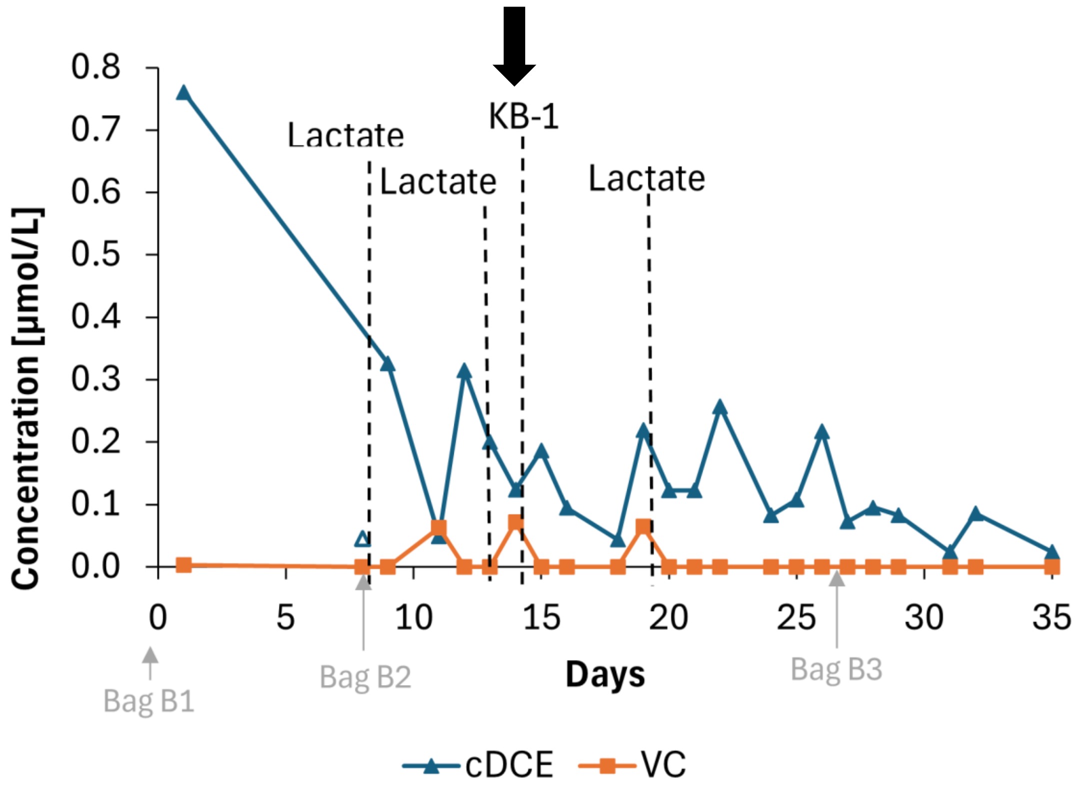
VFAs at the outlet



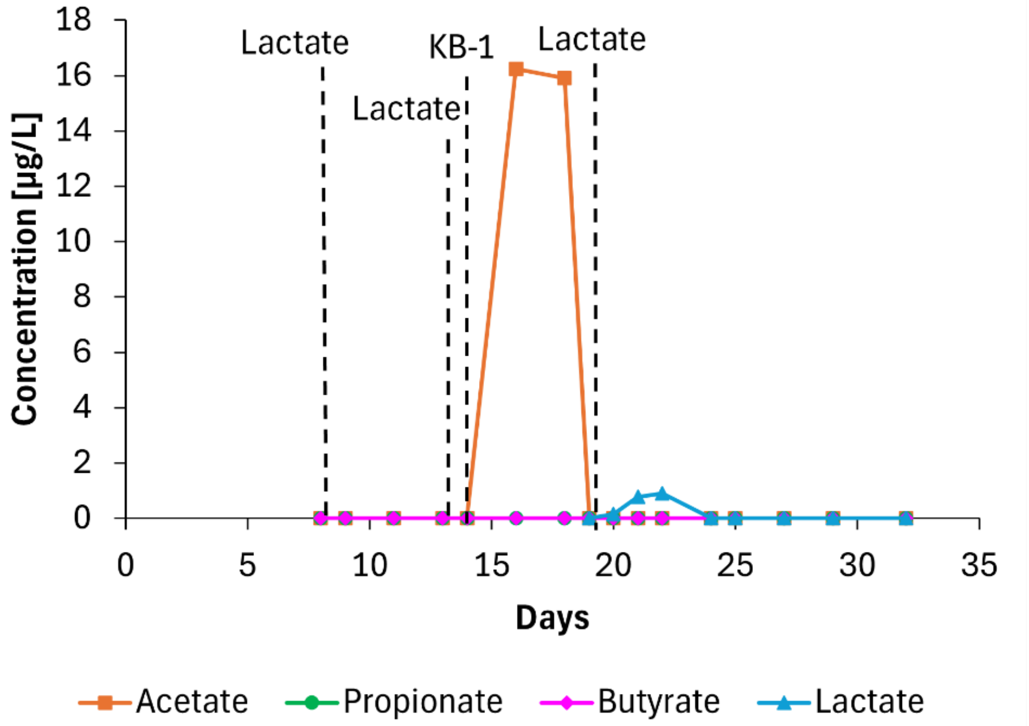
- Almost no VC → abiotic degradation
- Indigenous bacterial activity

Column B – Biotic

Chlorinated ethenes at the outlet



VFAs at the outlet



- Microbial activity

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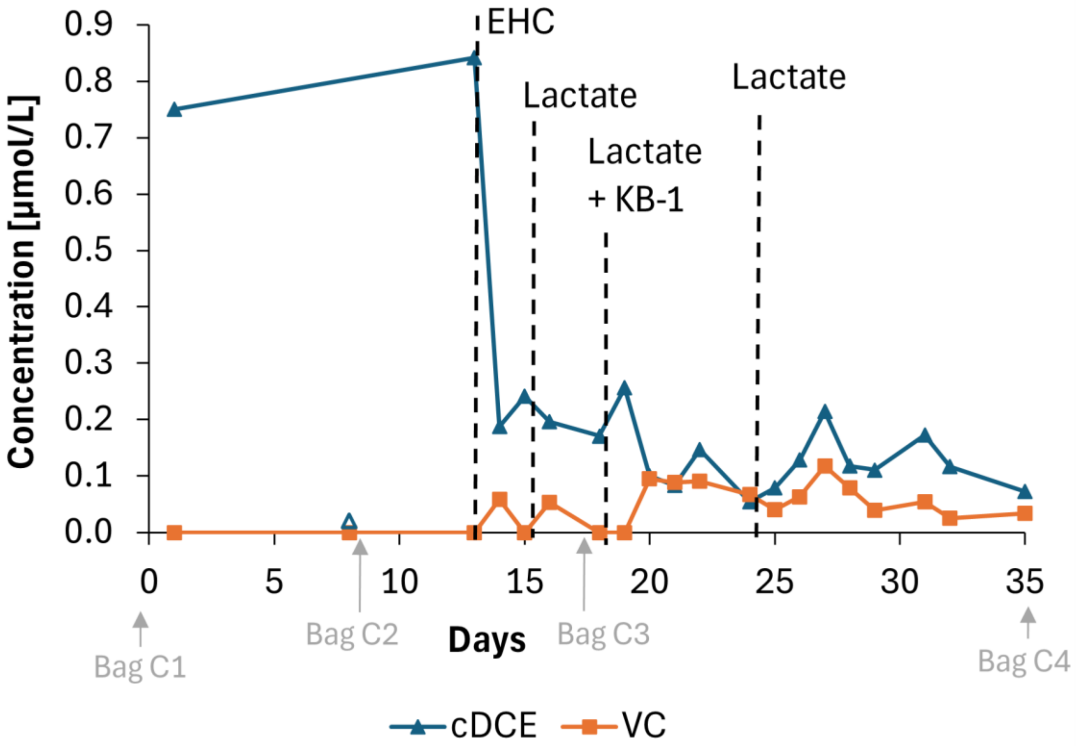
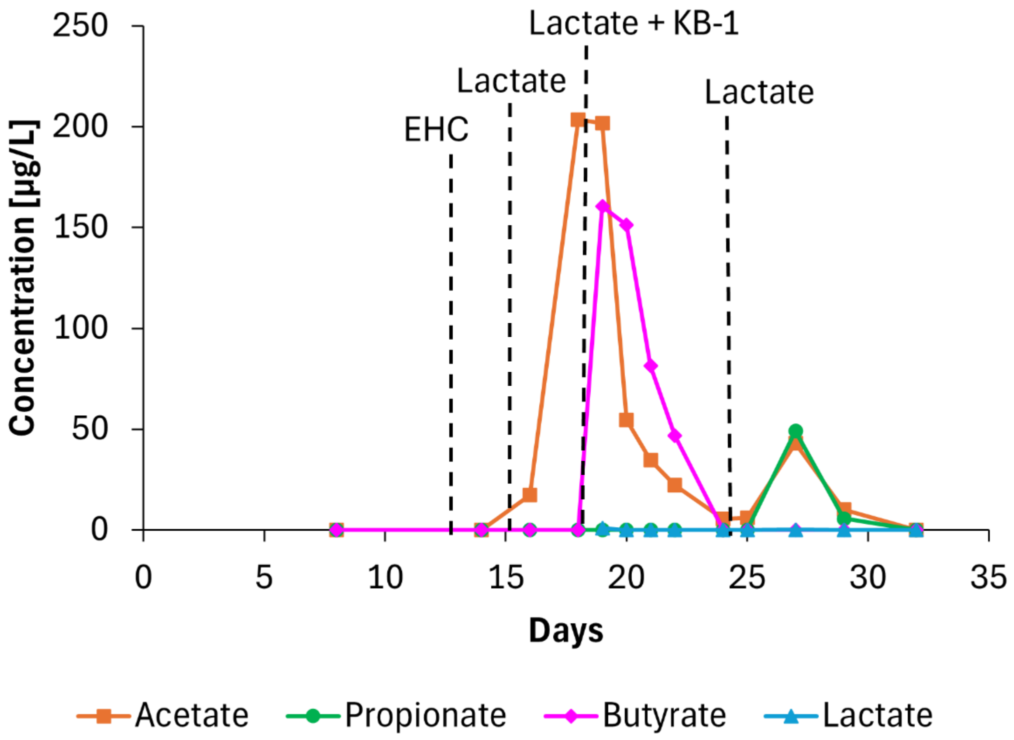
Column C - Combined

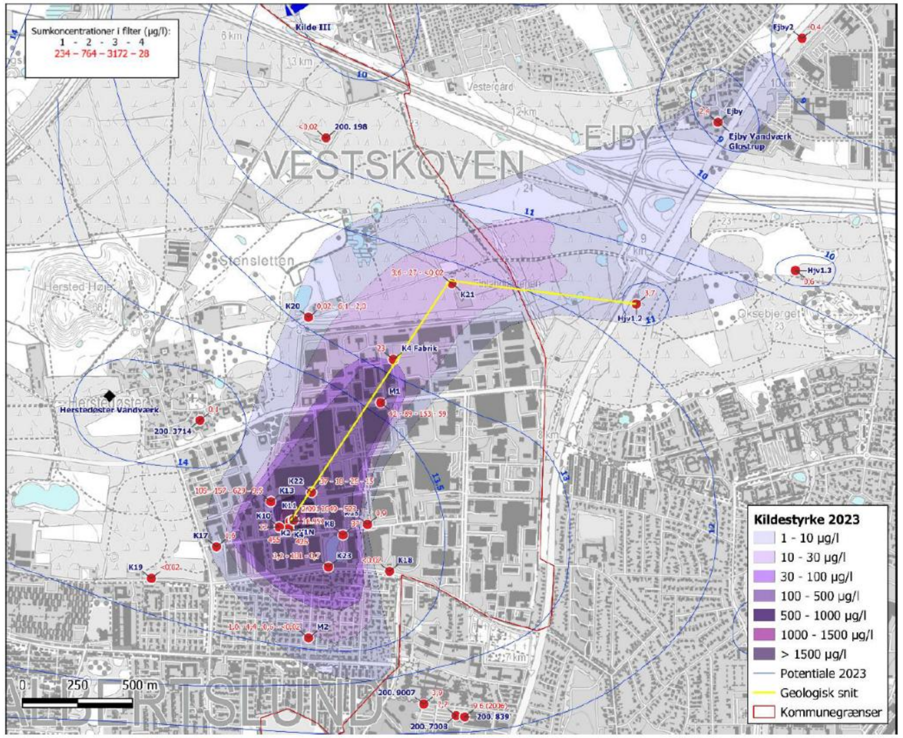
Chlorinated ethenes at the outlet



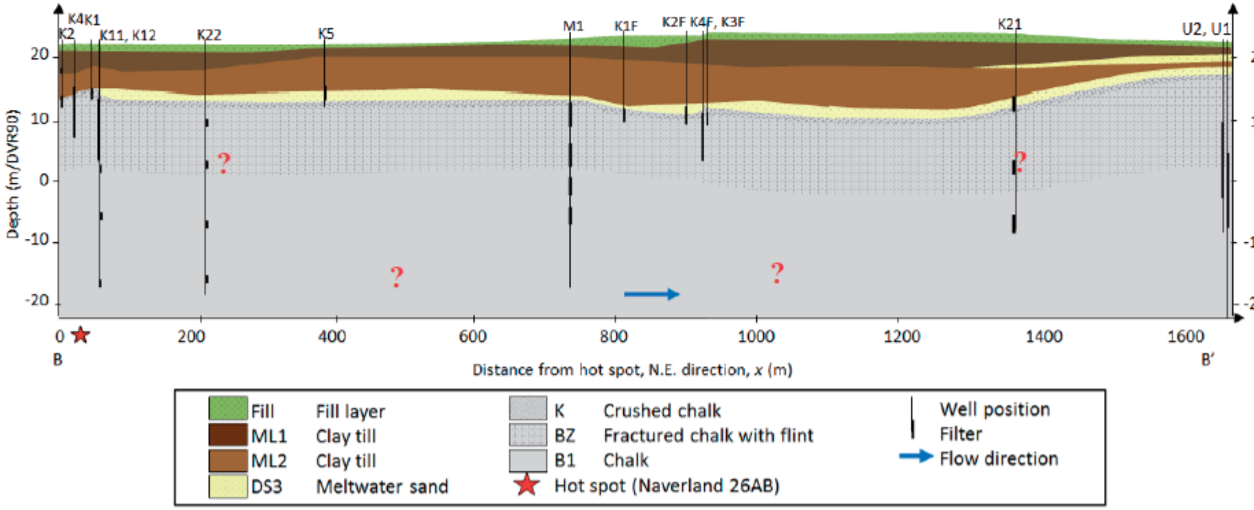
VFAs at the outlet



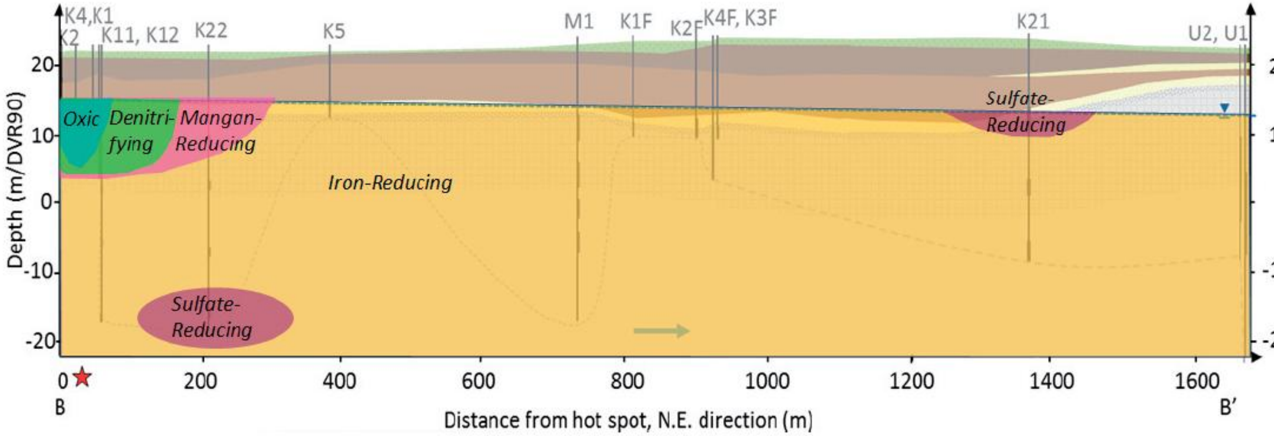
Naverland site



[HOFOR, 2024]

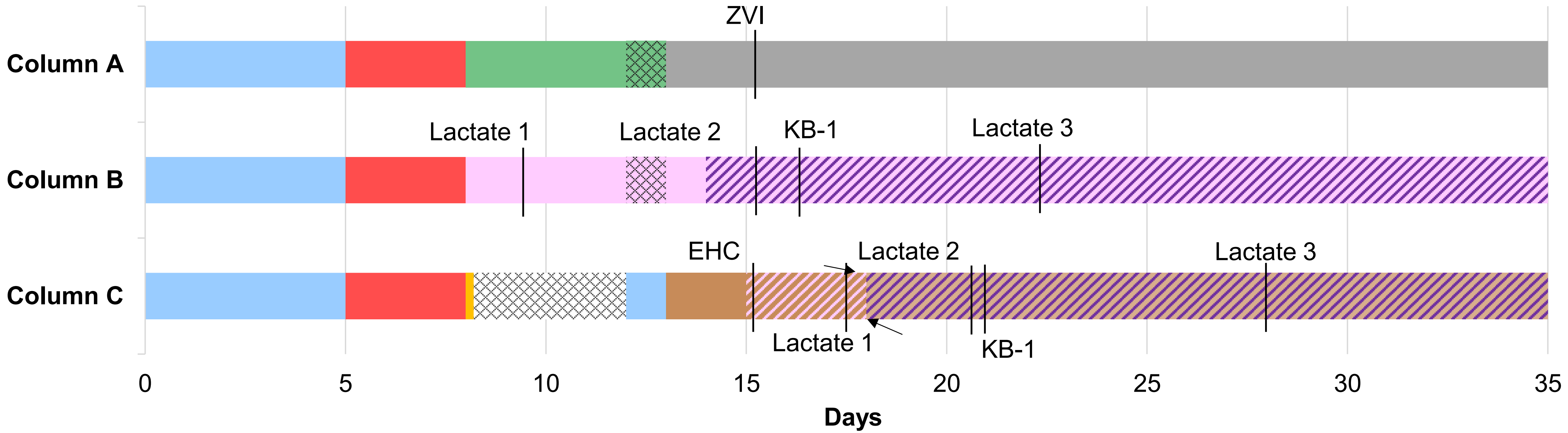


[Hemdorff, 2013]



[Hemdorff, 2013]

Chronology and amendments



- Saturation of the columns with groundwater
- Tracer test
- Natural attenuation in column A
- Flow stopped in columns A and B during resaturation of column C
- Chemical reduction with ZVI in column A
- Biostimulation with lactate in column B
- Biostimulation with lactate + bioaugmentation with KB-1 in column B
- Start pumping EHC Liquid in column C
- Column C clogged
- Chemical reduction with EHC in column C
- Chemical reduction with EHC + biostimulation with lactate in column C
- Chemical reduction with EHC + biostimulation with lactate + bioaugmentation with KB-1 in column C

Evonik products

OVERVIEW OF PRODUCTS

EHC® ISCR Reagent combines microscale zero-valent iron (ZVI) and organic carbon promoting both abiotic and biotic treatment mechanisms. It is the longest lasting of our product offerings and also offers the highest reducing power. EHC is the culmination of years of research in finding the optimal balance of reactivity and longevity.

EHC® Liquid Reagent is a combination of ELS™ Microemulsion and ferrous iron to provide both abiotic and biotic reduction of contaminants in an easy-to-use liquid form.

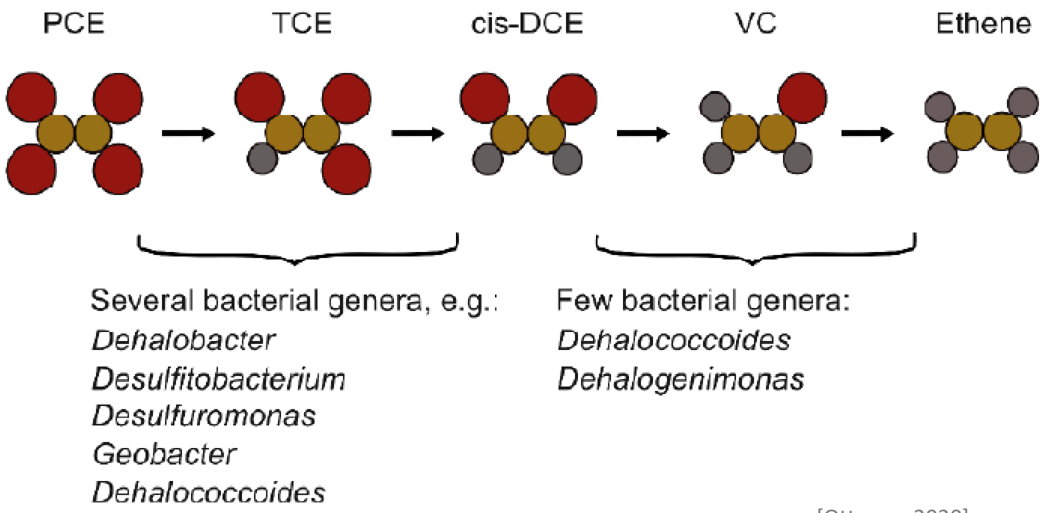
ELS™ Microemulsion is a 25% concentration lecithin microemulsion; a long-lasting, food-grade, liquid organic carbon substrate for enhanced reductive dechlorination / anaerobic bioremediation. ELS is also available as 100% ELS Concentrate that can be emusfied in the field.

Product	Composition	Mechanisms		
		Enhanced Reductive Dechlorination	Chemical Reduction	
			Direct	Indirect
EHC	• 60% Organic Carbon (plant particles) • 40% Micro-Scale ZVI	Yes	Yes	Yes
EHC Liquid	• Emulsified Lecithin Substrate • Soluble Organo-Iron Powder (Fe(II))	Yes	No	Yes
ELS	• Emulsified Lecithin Substrate • Availalble as 25% Microemulsion and 100% Concentrate	Yes	No	No

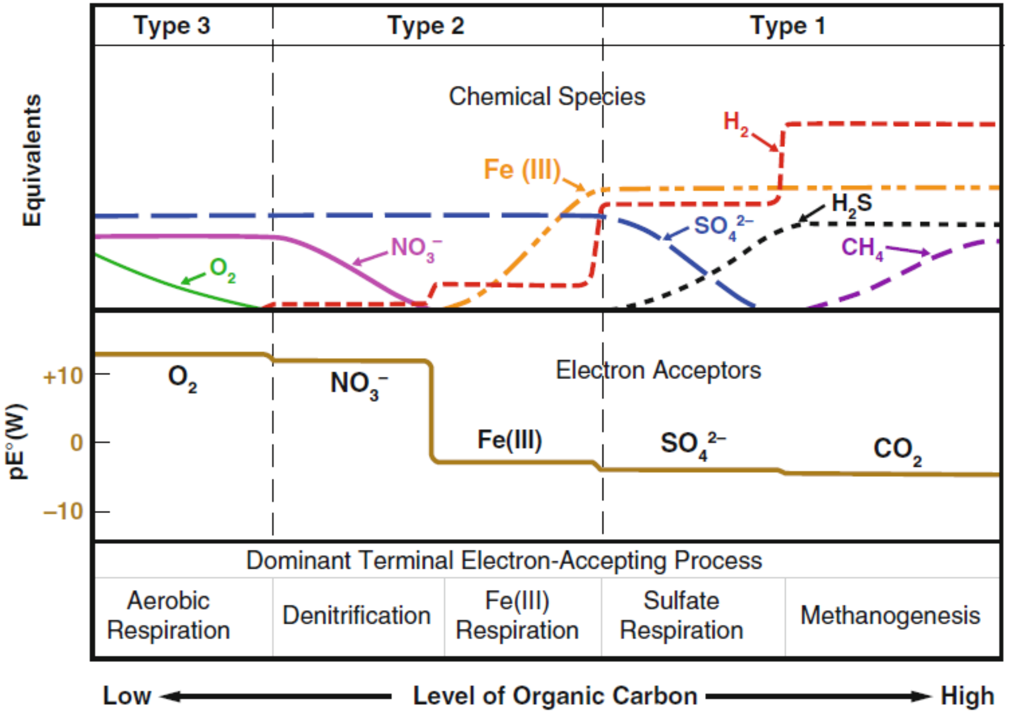
[Soil and Groundwater Remediation | EHC® Reagent - Evonik Industries]

DTU

Chlorinated ethenes – biodegradation



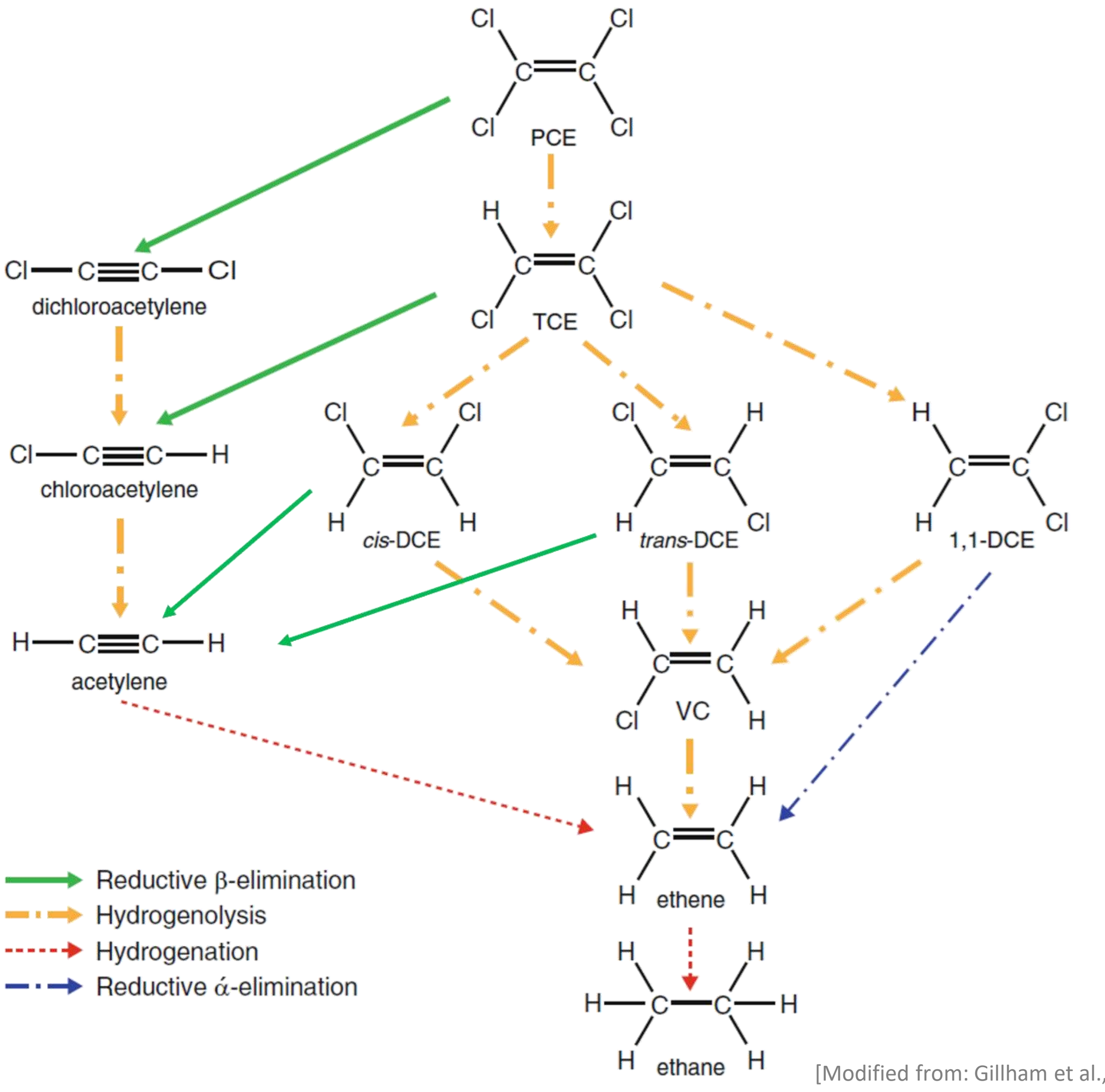
[Ottosen, 2020]



[Bouwer et al., 1984]

DTU

Chlorinated ethenes – degradation



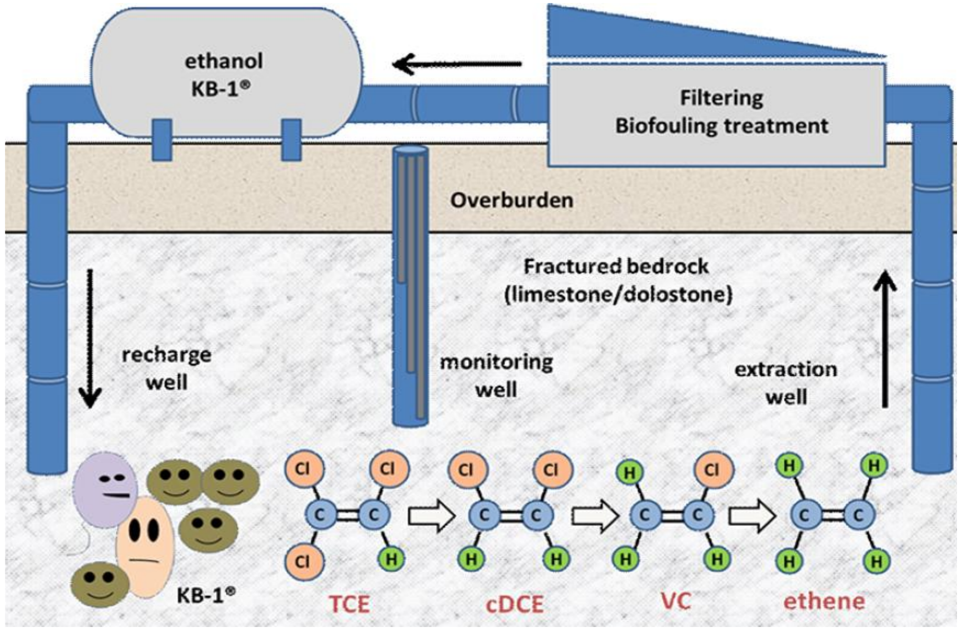
[Modified from: Gillham et al., 2010]

Potential for field applications

Application strategies

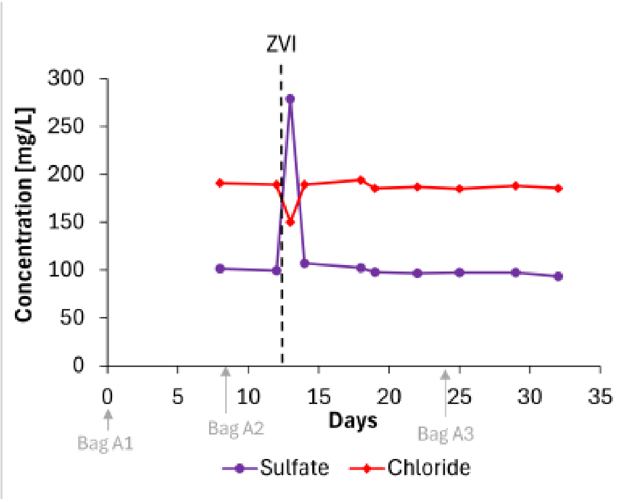
- ➢ Several push injections
- ➢ Recirculation of amendments

Recirculation scheme

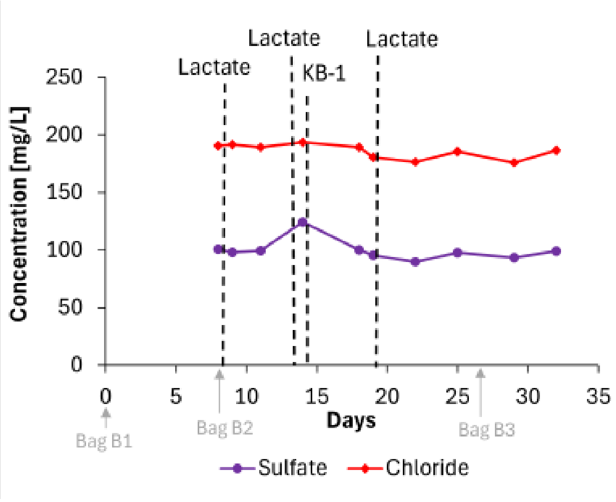


[Pérez-de-Mora et al., 2014]

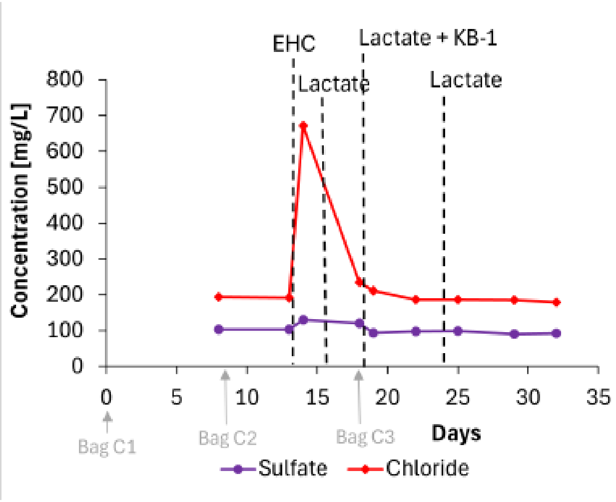
Anions



(a) Column A, amended with ZVI.



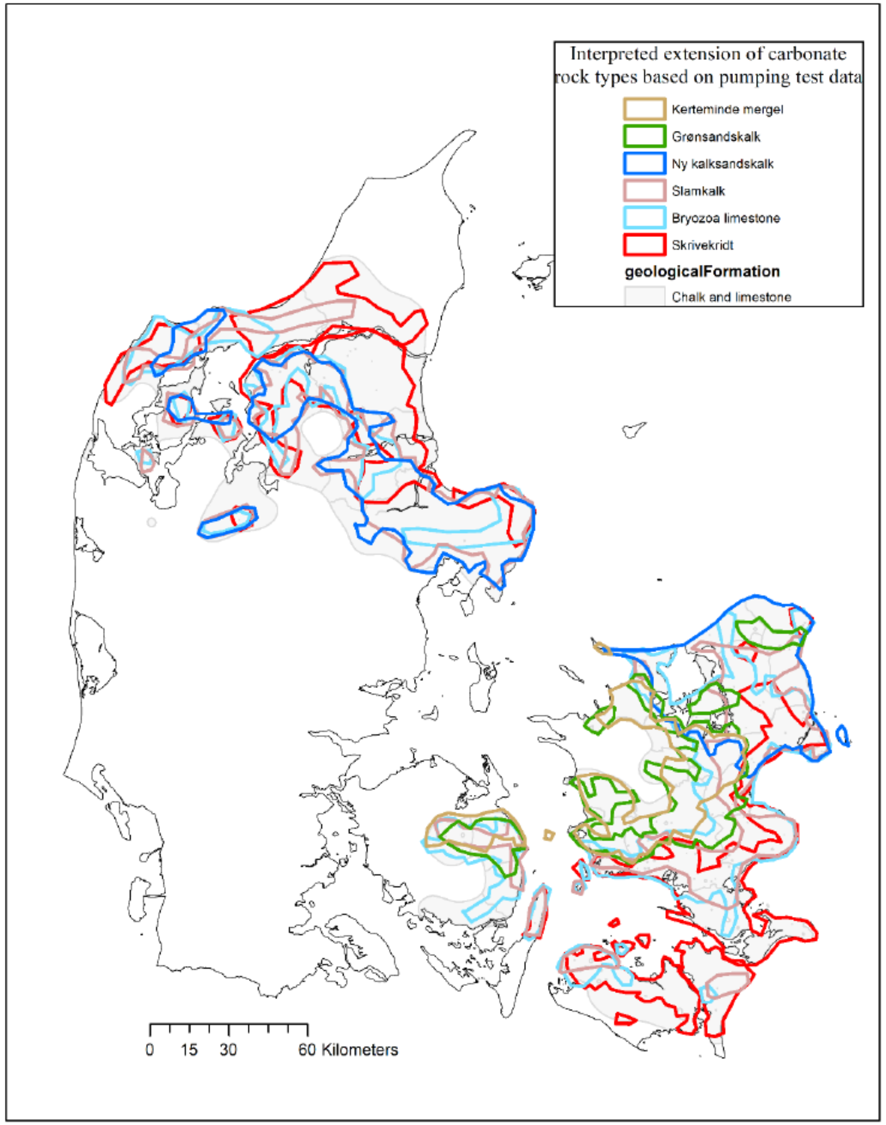
(b) Column B, amended with lactate and KB-1®.

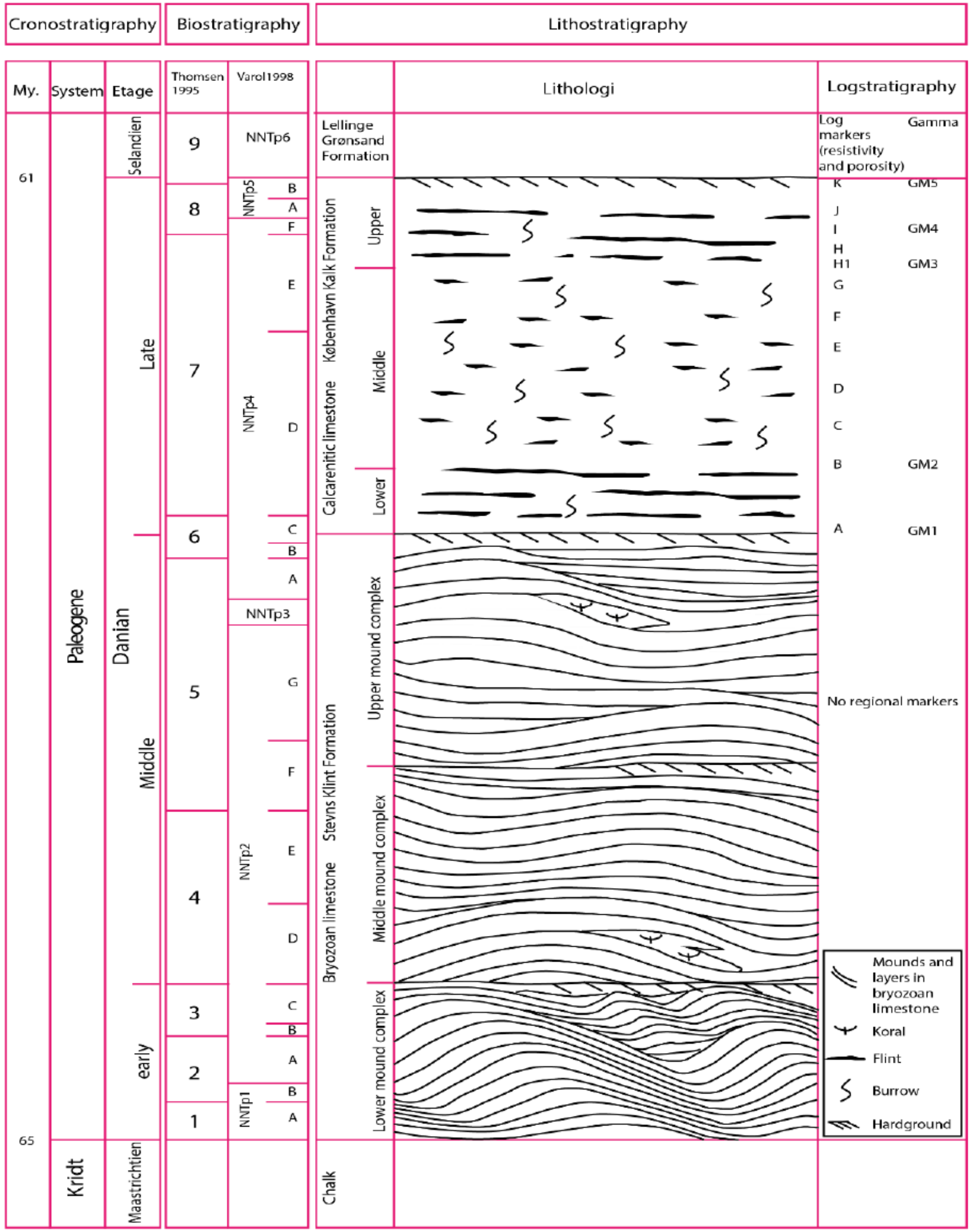


(c) Column C, amended with EHC®, lactate and KB-1®.

Figure 7.8: Sulfate [mg SO_4^{2-}/L] and chloride [mg Cl^-/L] concentrations measured at the outlet in columns A, B and C. Changes in Tedlar bags are indicated by the gray arrows below the x-axis. The quantification limit is 7.5 mg/L, both for sulfate and chloride.

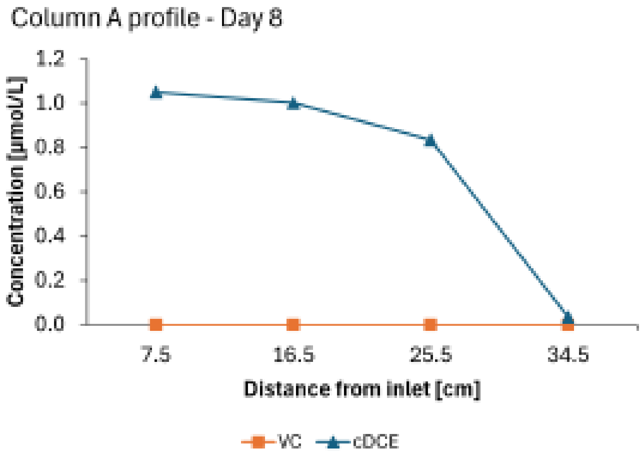
Limestone aquifers



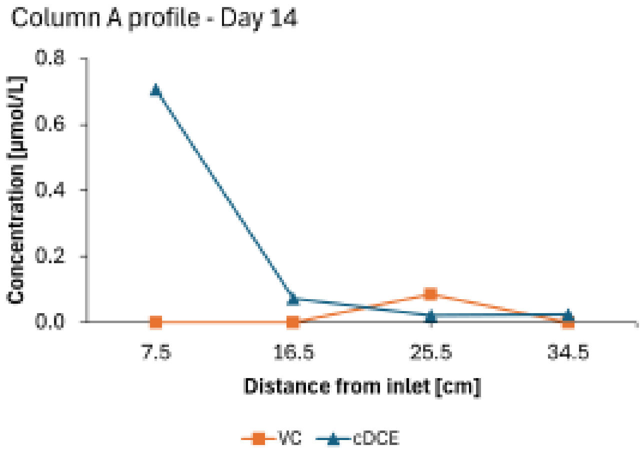


Column A

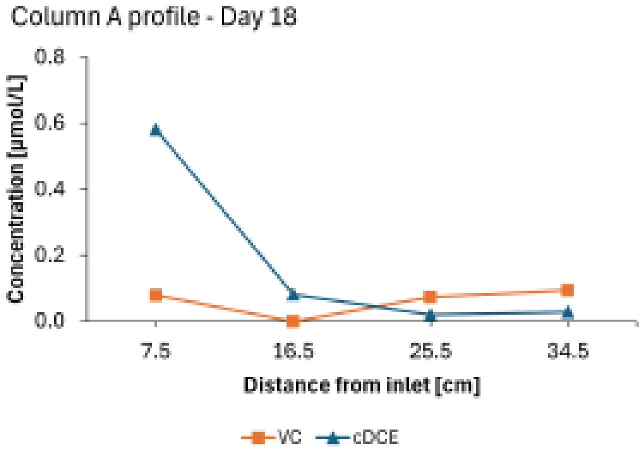
CE profiles



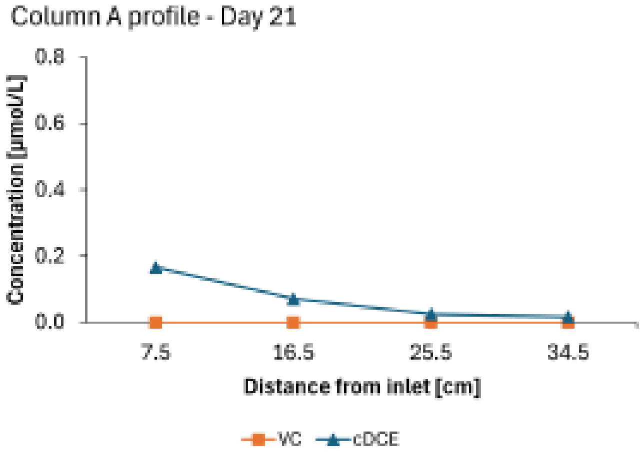
(a)



(b)



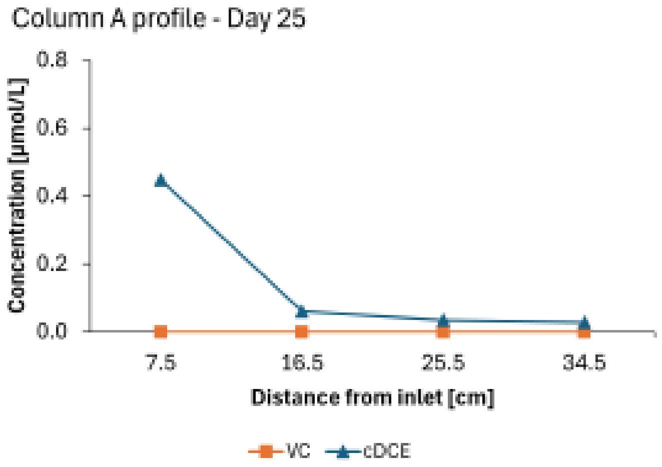
(c)



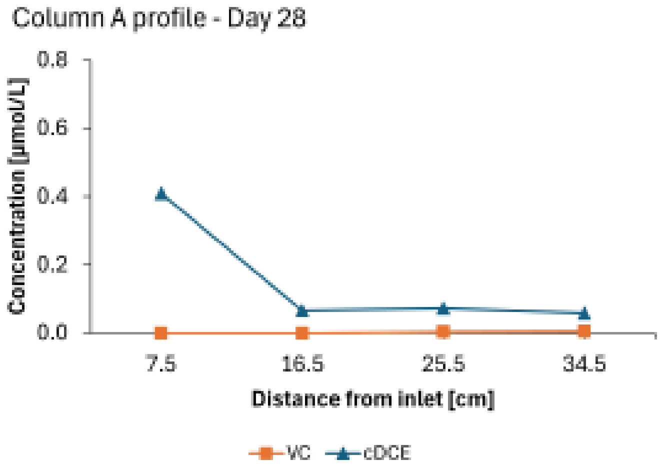
(d)

Column A

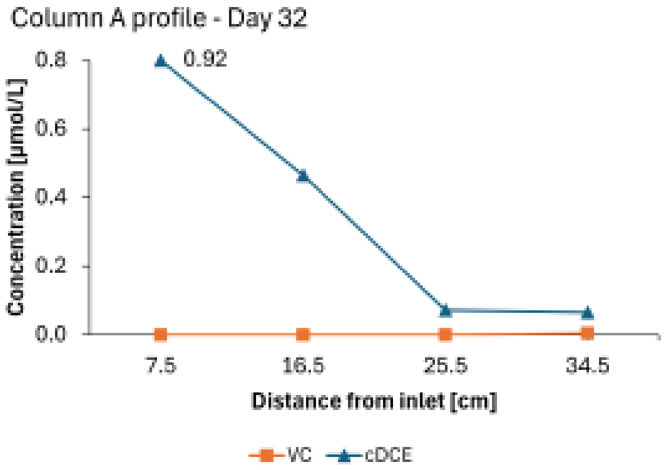
CE profiles



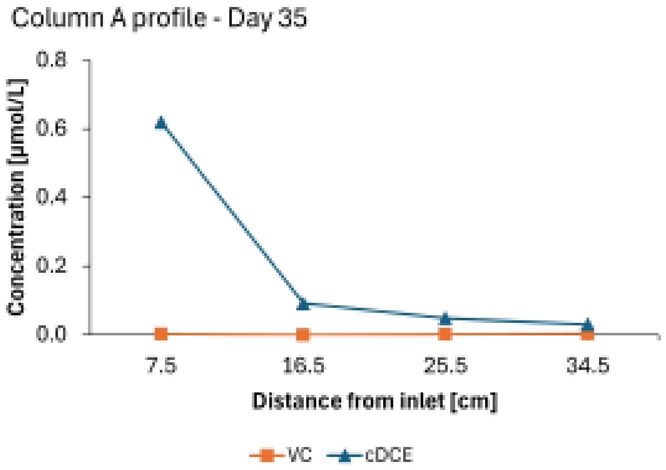
(e)



(f)

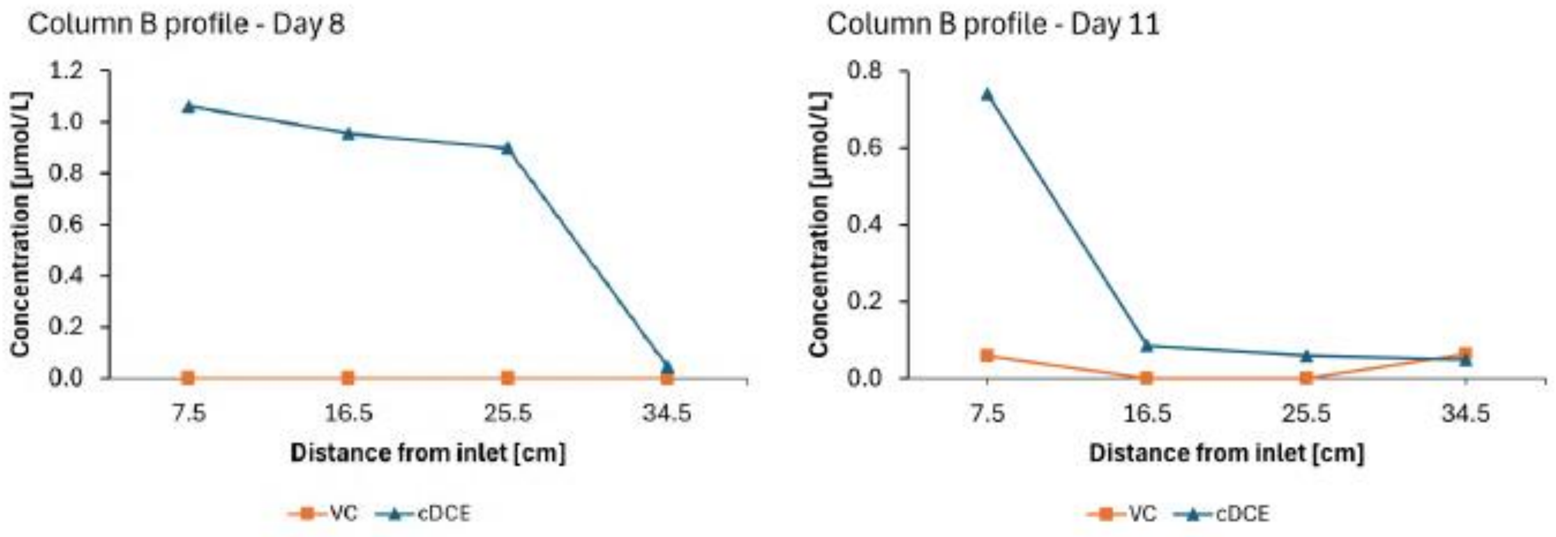


g)

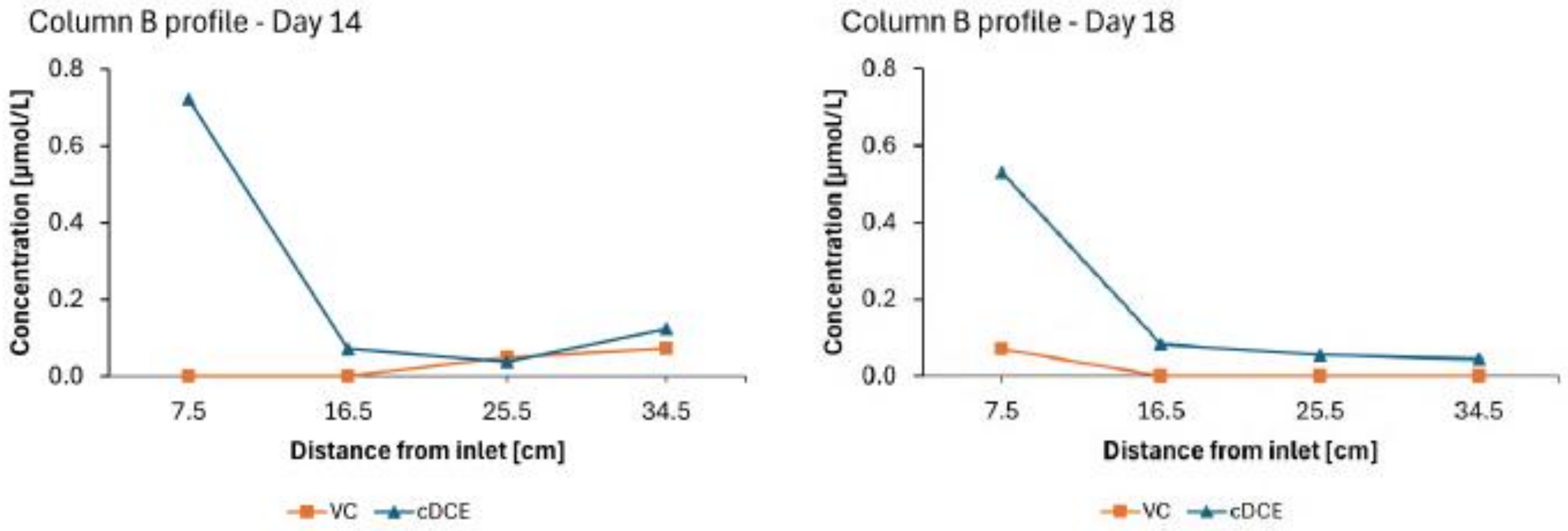


(h)

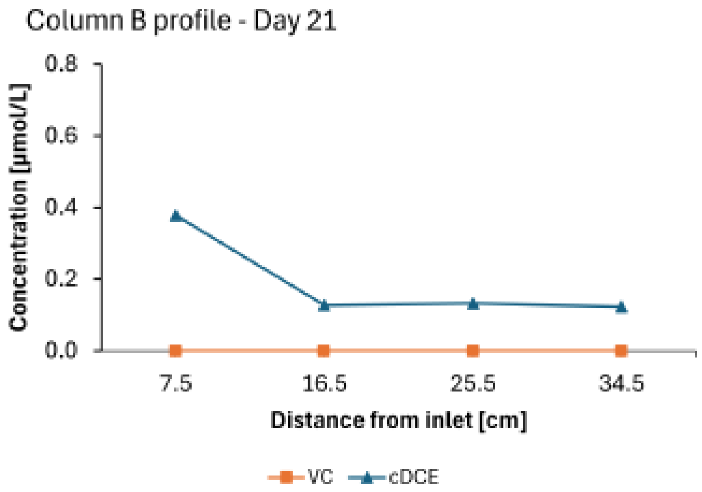
Column B
– CE profiles

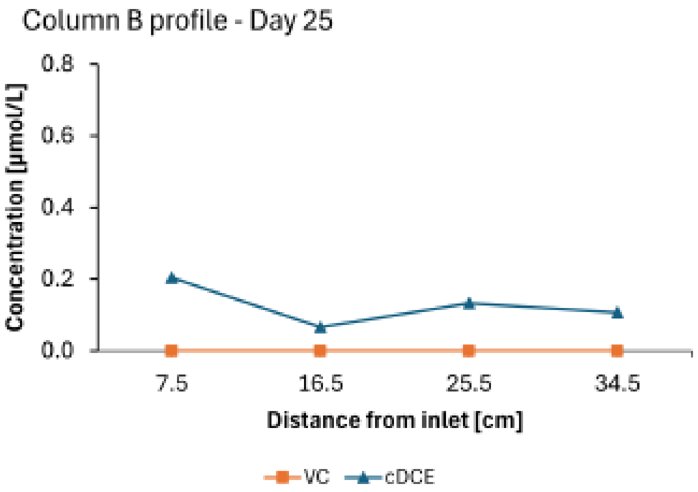


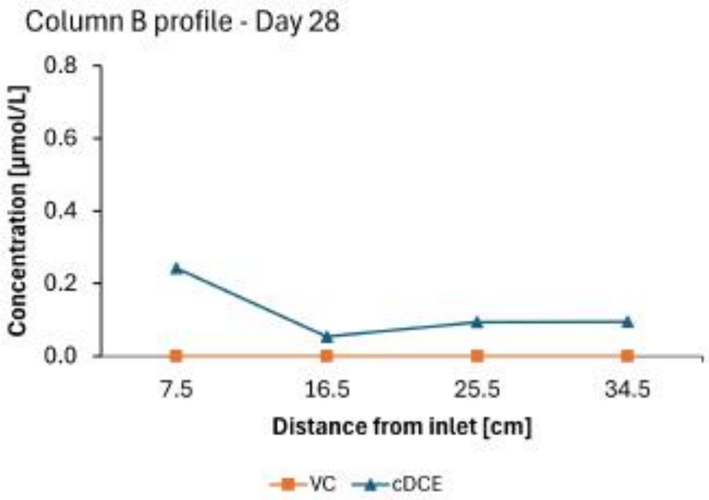
(a) (b)

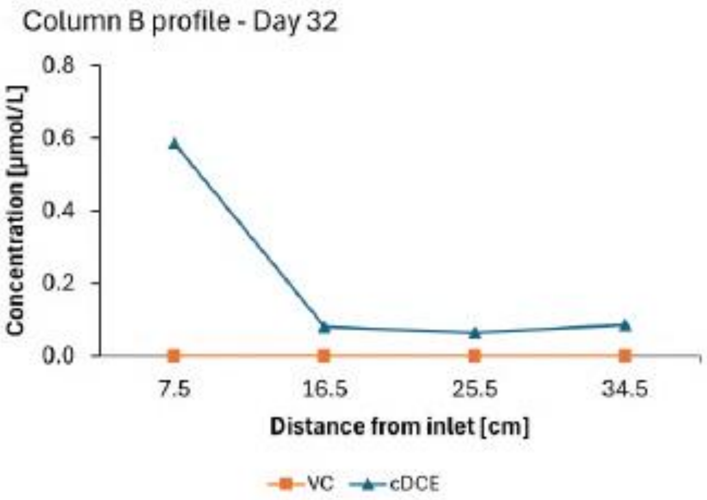


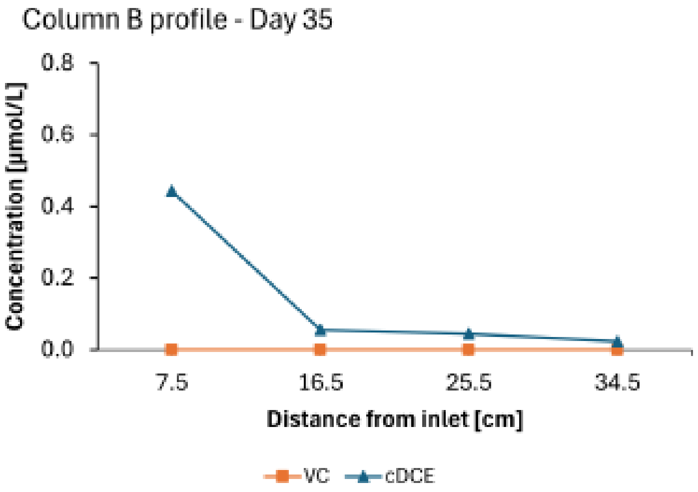
Column B – CE profiles



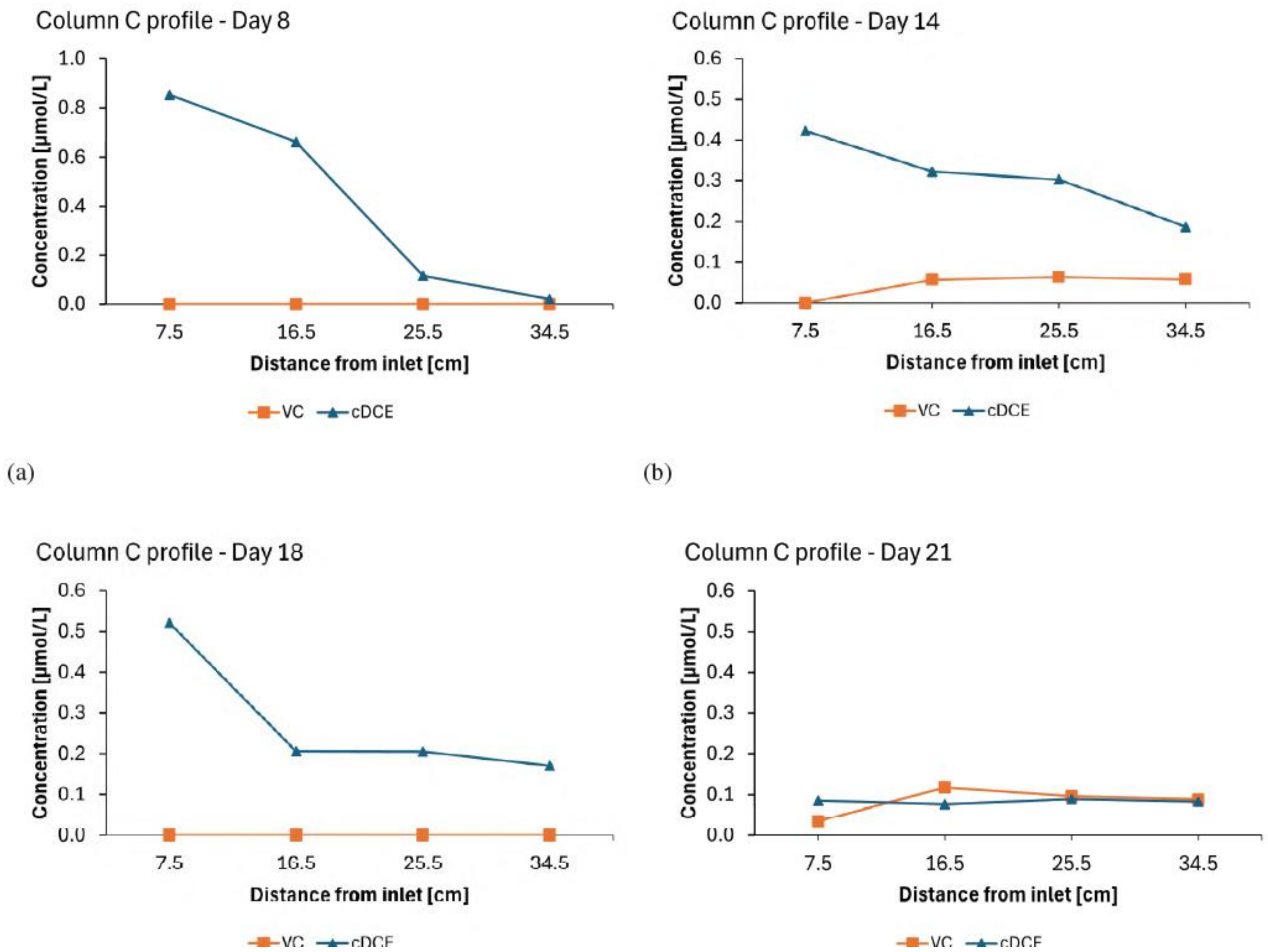




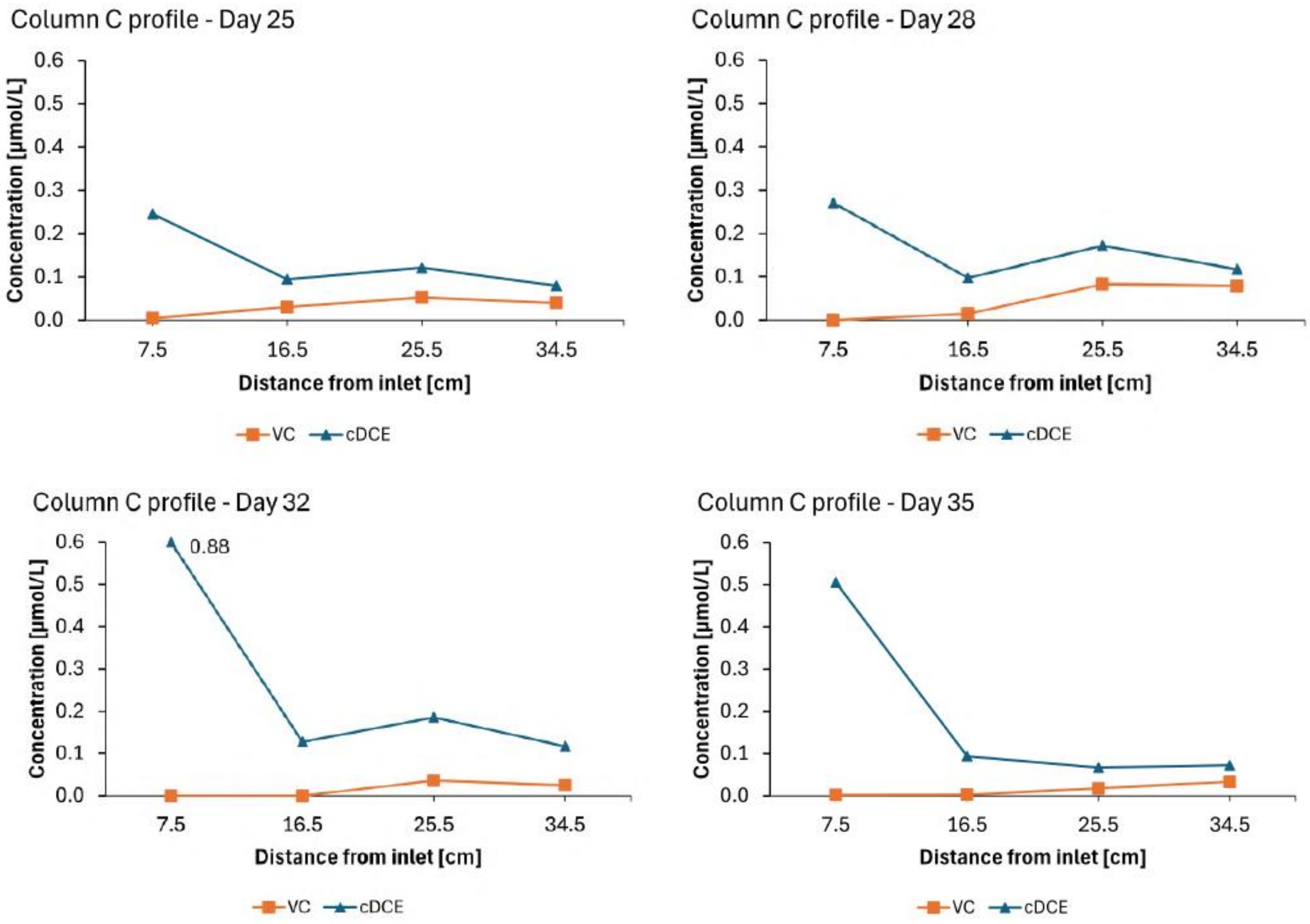




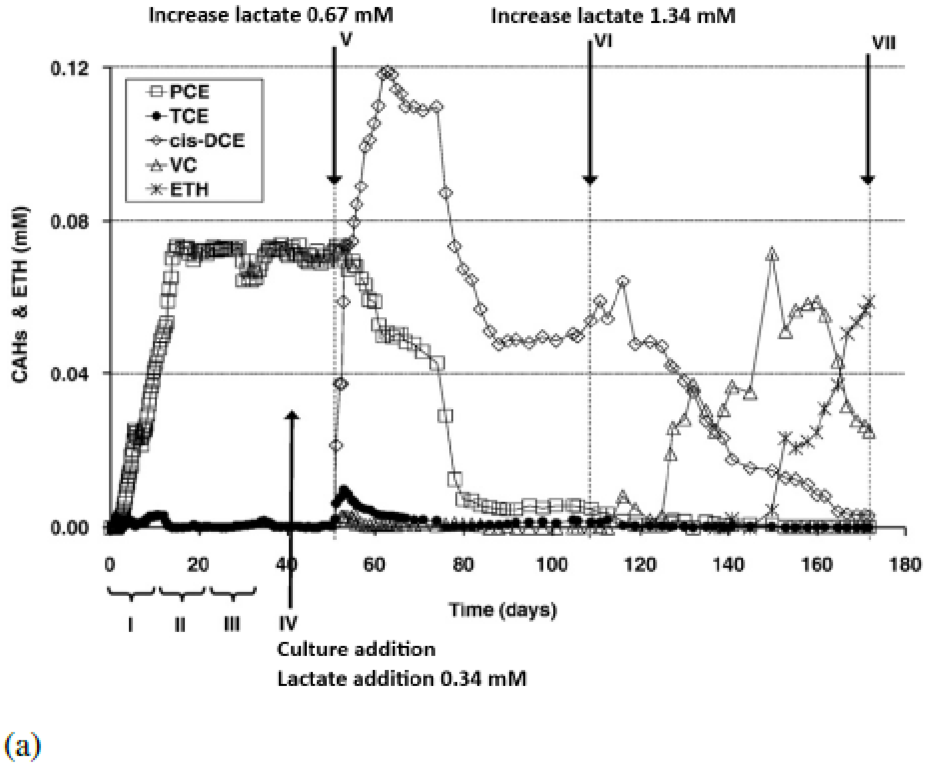
Column C
– CE profiles



Column C
– CE profiles



Lab studies



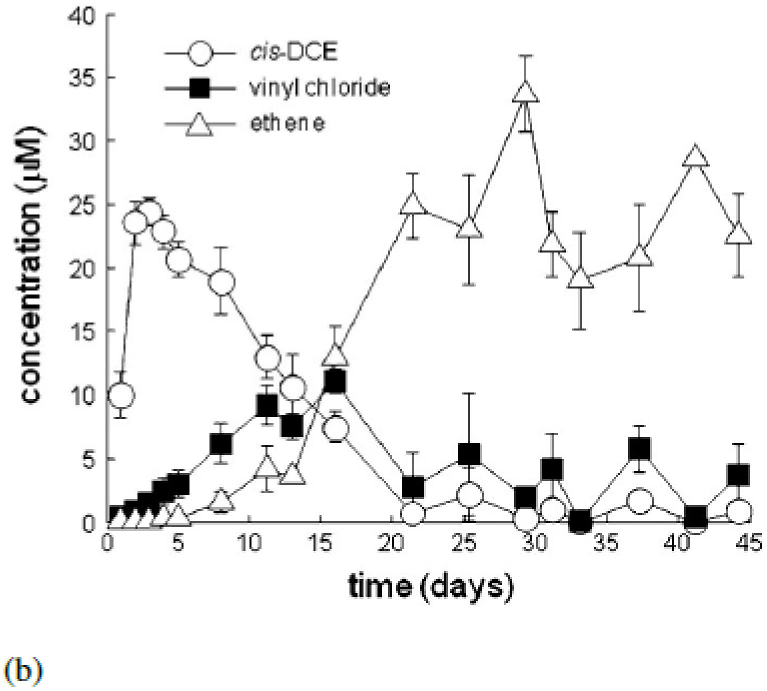
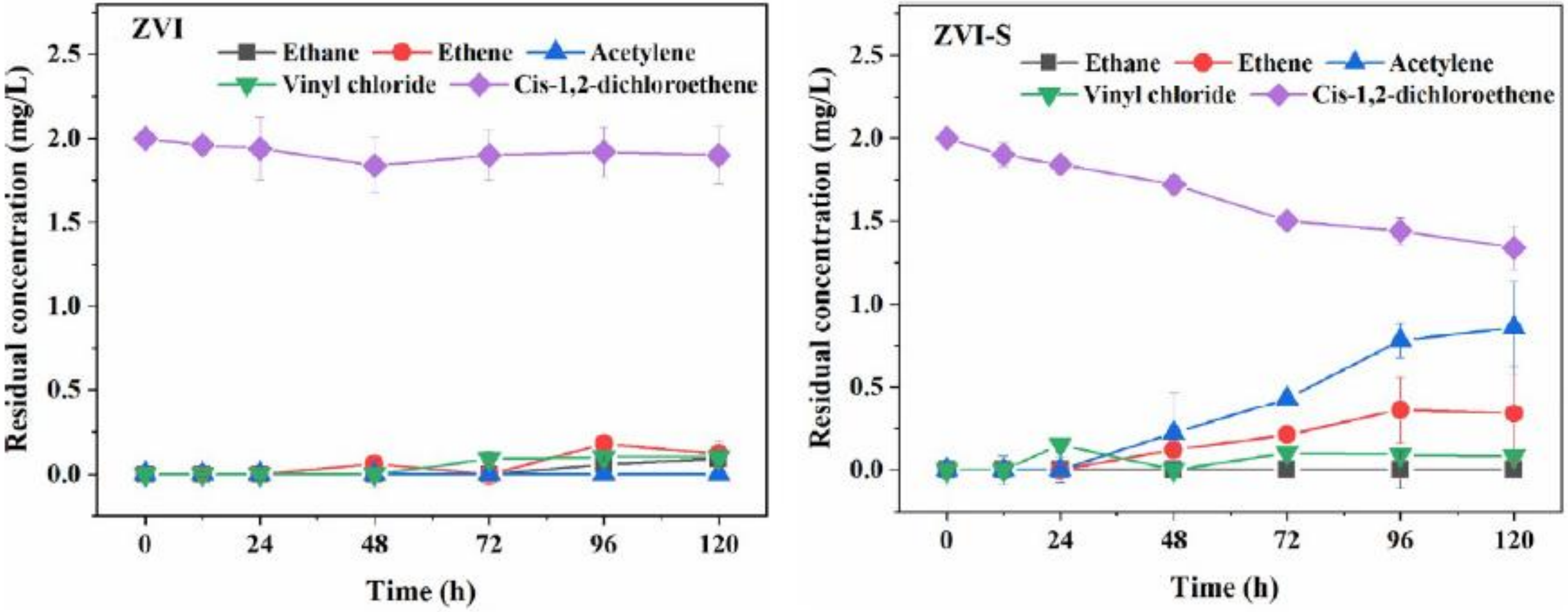


Figure 4.2: (a) Degradation of PCE to TCE, cDCE, VC and ethene during 170 days of column operation (adapted from Azizian et al. 2008), (b) Degradation of cDCE to VC and ethene during 45 days of column operation with a flow rate of 0.23 mL/min (Mendoza-Sanchez et al. 2010).

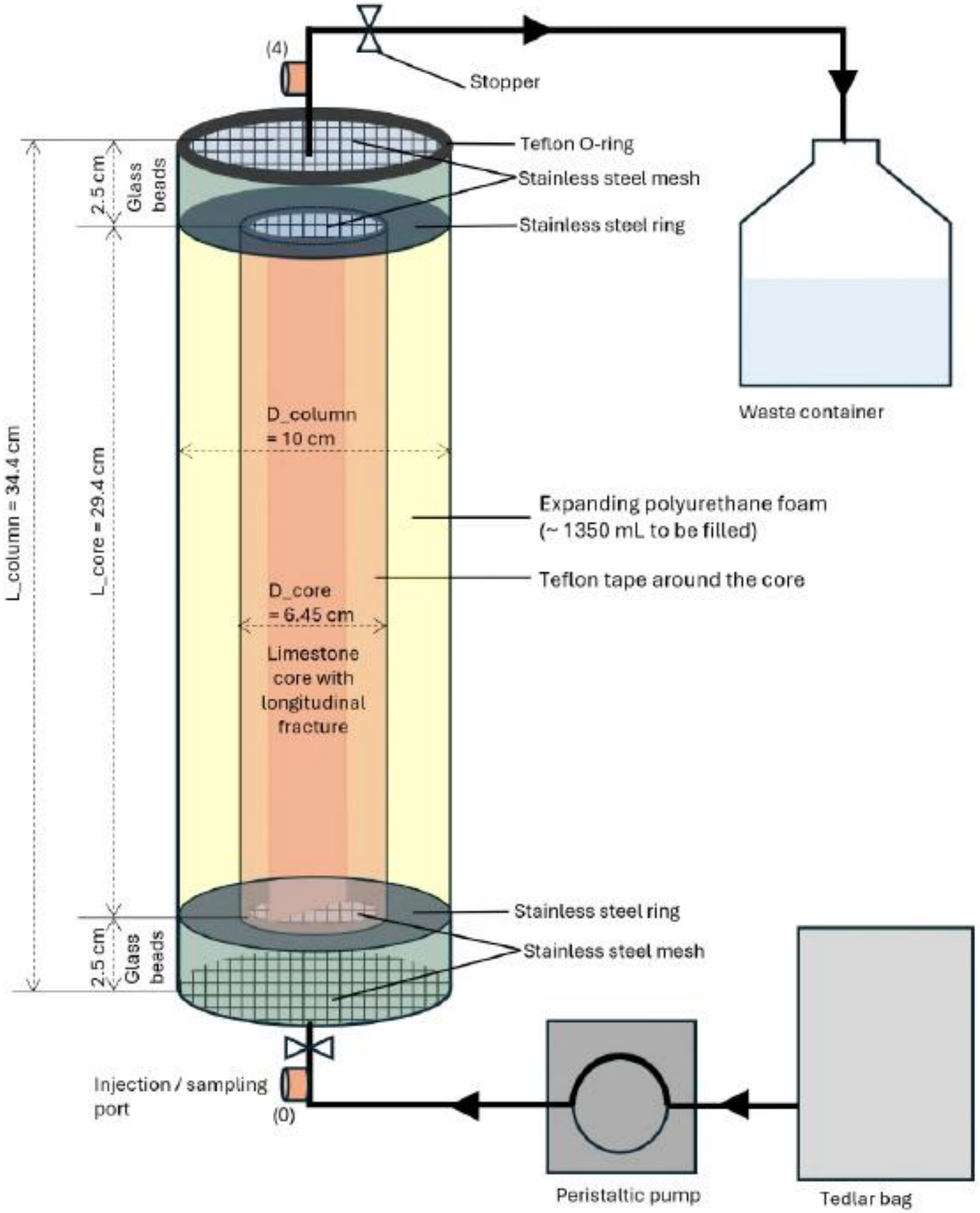
Lab studies – ZVI vs Sulfidated ZVI



(a) ZVI (b) Sulfidated ZVI

Figure 4.1: cDCE degradation by non-sulfidated ZVI (a) and sulfidated ZVI (b) (Qian et al. 2023).

Core column



(4)
Stopper
Teflon O-ring
Stainless steel mesh
Stainless steel ring
Waste container
2.5 cm
Glass beads
L_column = 34.4 cm
L_core = 29.4 cm
D_column = 10 cm
Expanding polyurethane foam (~ 1350 mL to be filled)
Teflon tape around the core
D_core = 6.45 cm
Limestone core with longitudinal fracture
Stainless steel ring
Stainless steel mesh
2.5 cm
Glass beads
Injection / sampling port
(0)
Peristaltic pump
Tedlar bag

GW extraction

Limestone collection